ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 491

THE INFLUENCE OF URAEMIA AND ELECTROLYTE DISTURBANCES ON MUSCLE ACTION POTENTIALS AND MOTOR NERVE CONDUCTION IN MAN

by

BOTTNARYD 1968

ACTA MEDICA SCANDINAVICA

has been published since 1919 as a continuation of Nordiskt Medicinskt Arkiv, founded in 1869 by Axel Key The first volume of Acta Medica Scandinavica is therefore numbered LII (52)

The chief editors have been Axel Key 1869—1900, C G Santesson 1901—1915 I Holmgren 1916—1957 and Birger Strandell 1958 to date

Acta Medica Scandinavica publishes original research papers in the field of internal medicine Priority will be given to authors from countries which are re presented on the editorial board Contributors from those countries should submit their papers to a representative of the country in question Contributors from other countries should send their papers to the Editor at the address below

Submission of a manuscript for publication will be held to imply that the paper has not been published elsewhere and that if accepted it will not be republished in any other journal in the same or similar form, without the Editor s written consent

Papers will be published in English, French or German at the authors choice followed by a summary in English

The manuscripts shall be in final form as submitted They shall be typewritten with double spacing and broad left hand margin

Subscription

The annual subscription to the journal covering two volumes each of 6 numbers • is 140 Sw crowns or U S $ 27 25 *including postage,* in the Scandinavian countries and in Holland 120 Sw crowns

Address for subscriptions and all communications

ACTA MEDICA SCANDINAVICA

Almqvist & Wiksell Gamla Brogatan 26, Stockholm C, Sweden

Requests for duplicate copies of numbers that have gone astray can only be considered if made within a fortnight of the receipt of the following number

ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 491

From the Medical Department B (Renal Clinic) University of Lund Sweden

THE INFLUENCE OF URAEMIA AND ELECTROLYTE DISTURBANCES ON MUSCLE ACTION POTENTIALS AND MOTOR NERVE CONDUCTION IN MAN

BY

TORE LINDHOLM

Bottnaryd 1968

I INTRODUCTION

Poor general condition tiredness and *weakness often imprint the clinical* picture of severe renal diseases In patients with renal insufficiency an affection of the central nervous system may be found (see e g Schreiner and Maher 1961), but sometimes also a muscular weakness of which a peripheral cause can be traced Various developments can be discerned clinically in a muscular weakness of this type A fairly acute form can be observed in patients suffering from both acute and chronic renal insufficiency in conjunction with disturbances in the electrolyte balance The sensitivity of the neuromuscular system to disturbances in the electrolyte environment is probably the cause in these cases of the muscular weakness which disappears when the electrolyte balance is corrected In other patients especially those with chronic renal insufficiency a more persistent form of muscular *weakness can sometimes be observed* The explanation for this may be either a primary myopathy or a neuropathy

The observation of neuropathy arising as a consequence of chronic diseases of the urinary tract is old (Charcot, 1880) Interest in neuropathies in patients with chronic renal insufficiency only developed however after Scribner et al (1960) had provided a clinically applicable technique for regular dialysis treatment By means of this technique patients without a renal function could be kept alive for long *periods and among them neuropathy was* often an obstacle to rehabilitation Subclinical neuropathies may also occur in uraemic patients In recent years there have been many reports of findings of reduced conduction velocity in both motor and sensory nerves in patients with chronic renal insufficiency some of whom have been on regular dialysis treatment (Asbury et al 1963 Funck Brentano et al 1964 Preswick and Jeremy 1964 Versaci et al 1964 Konotey Ahulu et al 1965 Tenckhoff et al 1965 Callaghan 1966 Dinapoli et al 1966 Forno and Alston 1967 Jebsen et al 1967 Nielsen 1967) Some of these (Asbury et al 1963 Dinapoli et al 1966 Forno and Alston 1967) also ncluded electromyographic examinations but none of them attempted to correlate the electromyographic findings with the *patients electrolyte condition*

Findings of myopathy in patients with chronic renal insufficiency have been reported by Merrill (1965) and Poinso et al (1965) These latter authors examined 30 patients with chronic renal insufficiency with biopsies from musc quadriceps of whom 10 were normal 15 had minimal or moderate changes and 5 showed pronounced changes which were interpreted as primarily myogenous and resembled those observed in certain other

chronic states such as cancer and malnutrition

In reports on muscular weakness due to electrolyte disturbances in patients with renal diseases the potassium ion occupies a central position both hyperpotassemia and hypopotassemia leading to muscle paralysis In 1915 Smillie described a patient with chronic renal insufficiency who after receiving 10 grammes of potassium chloride to induce diuresis complained of weakness abdominal distress and precordial pain Finch and Marchand (1943) reported two patients with renal insufficiency and potassium intoxication which led to flaccid quadriplegia and finally cardiac standstill Muscle paralysis in patients with renal insufficiency and hyperpotassemia has since then been reported by several authors (Finch et al 1946 Kolff 1950 Merrill et al 1950 McNaughton and Burchell 1951 Herman and Rado 1966) In some cases a muscular paralysis has also been described in patients with renal insufficiency and hypopotassemia (Brown et al 1944 Kolff 1950 Küchel et al 1951)

A low serum sodium concentration can accentuate the paralytic effect of an increased serum potassium concentration The two patients with hyperpotassemia described by Finch et al (1946) had a flaccid paralysis which receded under treatment with intravenous sodium chloride solution despite the fact that the concentration of serum potassium at the same time remained essentially unchanged These authors together with Merrill et al (1950) pointed out that the potassium sodium relation seemed to be significant for the presence of muscular paralysis

Electromyographic examination of patients with renal insufficiency with simultaneous determination of serum electrolytes seems to have hitherto only been carried out by Hausmanowa Petrusewicz et al (1962) who examined 26 patients with acute renal failure In 22 of the patients who had hyperpotassemia there was on the average a shortened mean duration of motor unit potentials in 12 cases low voltage curves at the effort recordings in 5 instances a great number of polyphasic units and in 6 a spontaneous activity However, no conclusions could be drawn from the results owing to the fact that the experimental conditions were of a very complex nature which did not permit the clear cut elimination of the effect of factors other than potassium disturbances on the electric activity of the muscle

The purpose of the present study was to attempt to describe how disturbances in the electrolyte fluid balance affect the neuromuscular function As many of the patients included in the examination had uraemia the problem of neuropathy was also touched on The intention was however not to ascertain the possible presence of polyneuropathy The methods used for the investigation were electromyography and motor nerve stimulation In uraemic patients a neuropathy clinically affects mostly the lower extremities In order to try to avoid as far as possible nerves and muscles affected by neuropathy the examinations were therefore carried out in the upper extremities To investigate the function of the motor unit electromyography in the brachial biceps muscle was chosen whereby the presence of any spontaneous activity pattern and amplitude at maximal voluntary contraction mean duration of motor unit potentials and incidence of polyphasic potentials were recorded Electromyographic abnorma

lities suggest a disorder of the neuro muscular system, their characters can not always be used to localize the damage and they must be interpreted with particular caution in metabolic dis orders Spontaneous activity with fibril lation potentials therefore need not be a sign of denervation nor need a re duction in the mean duration of motor unit potentials be a sign of myopathy but they may indicate other disturbances in the neuromuscular system (Buchthal 1962) The peripheral nerve function was investigated by measuring the motor nerve conduction velocity in the ulnar nerve in the forearm and the distal la tency under stimulation of the ulnar nerve at the wrist by recording the stimulation response in the abductor digiti quinti muscle The amplitude in this stimulation response was recorded under isolated stimulus to observe any fall in the number of active muscle fibres and under repeti tive stimulation to detect, if possible, disturbances in the neuromuscular trans mission At the same time the uraemia and electrolyte condition of the patients was recorded In some cases biopsy from the anterior tibial muscle was carried out to investigate the incidence of any general histological changes and also by deter mining the muscle water content to com plete the electrolyte balance

II MATERIAL

A PATIENTS

The material consisted of (a) 109 electromyographic examinations on 65 patients of whom 28 were examined at least twice, and (b) 90 examinations with motor nerve stimulation on 55 of the patients All the patients were treated at Medical Department B (Renal Clinic) Lund and examined on the grounds of a known or suspected electrolyte disturbance and/or uraemia Figure 1 shows that the ages of the 65 patients were fairly evenly distributed between 20 and 70 and that 29 of the patients were women and 36 men Table 1 shows the patients diagnoses and the presence of reduced normal and increased concentrations of non protein nitrogen, plasma sodium plasma potassium and standard bicarbonate at the first electromyographic examination The diagnosis groups acute renal failure and primary pyelonephritis were the largest each covering 18 patients while the remaining 29 patients were distributed among the 8 other groups Besides a renal disease 4 patients had diabetes mellitus of whom only one had been treated with insulin earlier One of these patients also had a malignant neoplasm which also occurred in a further 3 patients At the first electromyographic examination 23 patients had a high uraemia with the non protein nitrogen concentration 200 mg per cent or higher Thirteen patients had a pronounced hyperpotassemia with the plasma potassium concentration 6 0 mEq per litre or higher and 19 patients had a metabolic

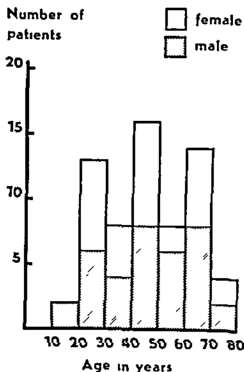


Fig 1 Age and sex distribution among the 65 patients studied

acidosis with the standard bicarbonate concentration 15 0 mEq per litre or lower The general condition of the patients at the first examination could be described

Table 1 The distribution of patients with regard to the diagnosis and presence of reduced normal and increased concentrations of non protein nitrogen plasma sodium plasma potassium and standard bicarbonate

Diagnosis		Number of patients	Non protein nitrogen mg per cent		Concentrations at the first electromyographic examination: Plasma sodium mEq per litre			Plasma potassium mEq per litre			Standard bicarbonate mEq per litre		
			20—45	> 45	< 131	131—141	> 141	< 3 3	3 3—4 4	> 4 4	< 21 3	21 3—24 8	> 24 8
Acute renal failure complicating	surgical diseases	11	—	11	8	3	—	1	—	10	7	2	1
	internal diseases	7	—	7	4	2	1	1	—	6	6	—	1
Primary pyelonephritis		18	1	17*	6	10	2	1	8	9	15	1	2
Secondary pyelonephritis		2	—	2	1	1	—	—	—	2	2	—	—
Subacute glomerulonephritis		3	—	3	1	1	1	1	—	2	1	1	1
Chronic glomerulonephritis		6	—	6**	1	4	—	—	2	3	4	1	—
Collagenous renal diseases		4	2	2	—	3	1	1	—	3	2	1	1
Miscellaneous renal diseases		7	3	4	2	3	2	—	6	1	2	3	1
Postrenal uraemia		2	—	2	—	2	—	—	1	1	1	—	—
Hyperparathyroidism		2	2**	—	—	2	—	2	—	—	—	—	1
Electrolyte disturbances without signs of renal disease		3	2	1	—	2	1	3	—	—	—	1	2
Total		65	10	55	23	33	8	10	17	37	40	10	10

* and ** in respectively one and two cases expressed as urea N mg per cent

as good in 6 cases slightly to moderately affected in 43 cases and bad in 16 cases

Eleven patients had previously received treatment with the artificial kidney but the interval between that treatment and the first electromyographic examination was such that no post treatment dis equilibrium was considered to remain At the time of the first examination none of the 65 patients was on regular dialysis treatment Examinations were carried out on 17 patients immediately before and after a treatment with the artificial kidney on 19 occasions The diagnoses in these cases were acute renal failure compli cating surgical diseases (2 patients) and internal diseases (3) primary pyelone phritis (5) subacute glomerulonephritis (2) chronic glomerulonephritis (1), col lagenous renal disease (1), postrenal uraemia (1) and miscellaneous renal dis eases (2) The medicine treatment of the patients was very varied and included over 60 different preparations most of which were only given to individual pa tients A more detailed analysis of the medicine treatment was not carried out The most common preparations were vita mins B and C, which were given to 42 patients (65 per cent) and peroral alu minium hydroxide which was given to 43 patients (66 per cent) Forty one patients were treated with one or more antibiotics the most common of which was chlor amphenicol (23 patients 35 per cent) No patient was treated with nitrofuran toin

The following three case histories will serve to further illustrate the data used

EMG 212 A 48 year-old woman who 15 years before had undergone laparatomy for a tumour of the uterus She fell ill on 10th November 1963 with a slight increasing pain in the lower part of the abdomen On 12th November vomiting began and she was taken into hospital on 13th Her general condition was fairly unaffected and her abdomen was distended but soft An X ray showed signs of a moderate mechanical ileus On the same day a laparotomy operation was performed to remove adhesions between the small in testines and the uterus During the dissection of the small intestine adhering to the per itoneum a perforation was obtained through which a considerable quantity of intestinal content was drained After careful cleaning of the abdomen the perforation was sutured When the small intestine was freed it had a rough slightly bleeding surface over an area of about 15 cms long This part of the small intestine was resected with a triangular ex cision of the mesentery and end to end anasto-mosis During the postoperative period the patient s blood pressure fell and oliguria de veloped She was therefore transferred to the renal clinic on the 8th postoperative day On arrival she was in a distressed con dition but was able to answer adequately and did simple mathematical calculations Her abdomen was soft but with a mo derate diffuse soreness Her reflexes were nor mal and the contraction force in the flexor muscles of the elbow was good The electro-myographic examination was carried out on the day of arrival when the patient had in addition to an extreme uraemia a hyperpotas semia and metabolic acidosis The patient was then treated four times with the artificial kid ney However her condition got gradually worse and she died 14 days after the operation An autopsy revealed a purulent peritonitis but without signs of suture insufficiency and renal tubular necrosis

EMG 142 A 34 year old woman who had earlier attempted to commit suicide and had been periodically treated in a mental hospital On 23rd June 1963 she took four teaspoons of a herbicide (sodium chlorate) with intent to commit suicide During the following five days she suffered abdominal pains vomiting and repeated loose evacuations She was taken to hospital on the fifth day and transferred to the renal clinic owing to oliguria and in creasing uraemia the following day The patient was a little listless but could answer questions adequately and her general condition was relatively lightly affected On the 9th day

she was treated with the artificial kidney when the concentration of non protein nitrogen had risen to 189 mg per cent and that of plasma potassium to 5 0 mEq per litre Electromyographic examinations were carried out before and after this treatment and again on the 11th day The patient then received a further treatment with the artificial kidney after which the urine production recommenced On the 40th day she was returned to the local hospital Endogenous creatinine clearance was then 51 ml per min

EMG 289 A 42 year-old woman who had had repeated attacks of infections of the urinary tract since 1950 For several years she had also had headaches which had caused consumption of analgesics containing caffeine phenacetin and phenazone Despite repeated cures with antibiotic treatment her renal function gradually deteriorated In 1957 the non protein nitrogen concentration was 44 mg per cent and endogenous creatinine clearance 60 ml per min In 1962 the non protein nitrogen concentration was 70 mg per cent In December 1963 when the patient was admitted to the renal clinic for the first time the non protein nitrogen concentration was 114 mg per cent and endogenous creatinine clearance 3 ml per min The kidneys were bilaterally reduced Despite conservative measures the renal function deteriorated further during the following months and the first treatment with the artificial kidney was carried out on the 13th March 1964 when the non protein nitrogen concentration had risen to 252 mg per cent and there was a considerable metabolic acidosis An electromyographic examination was carried out before and after this treatment Despite her affected condition the patient had a good muscular force A biopsy from the anterior tibial muscle at the same time showed a histologically normal condition The patient then went on to regular dialysis treatment and is still alive four years later

B BLOOD ANALYSES

Samples of blood were taken just before or soon after (0—4 hours) the electromyographic examinations In cases with a tranquil development isolated analysis results have been included from samples taken on a day other than that of the examination, such as, for example a normal non protein nitrogen value taken on some day before or after the electromyographic examination The analyses of the samples were included in the routine work at the Department of Clinical Chemistry, University of Lund (Head Prof C G Holmberg) Table 2 shows the methods and the normal range of blood analyses and their mean values at the first electromyographic examination of the patients The electrolyte concentrations in the extracellular fluid can be calculated by means of these values The electrolyte concentration in the plasma water and a Donnan factor for calculating the distribution of the electrolytes between the plasma and extracellular fluid must then be taken into account Thus the chloride concentration in the extracellular fluid, $[Cl]_e$, for example, can be calculated according to the following formula (Bergstrom 1962)

$$[Cl]_e = [Cl]_s \quad \frac{1000}{0\ 96\ (984—7\ 18\ [prot])},$$

where $[Cl]_s$ symbolizes the concentration of serum chloride and [prot] the concentration of serum protein in g per cent For the average serum protein concentration of 6 04 g per cent the correction factor was 1 107 The correction factor for the highest concentration of serum protein at 8 6 g per cent was 1 129 and for the lowest at 3 4 g per cent 1 086 These two correction factors do not deviate more than 2 0 per cent from the average If the average correction factor had been used in all cases there would have been an error which in extreme cases would have been 2 0 per cent at the most

Table 2 Methods and normal range for blood analyses and their mean values at the first electromyographic examination of the 65 patients

	Number	Mean value ± S E	S D	Normal range	Method
Non protein nitrogen mg per cent	59	170 ± 11	83	20 — 45	according to Rappaport (1949)
Plasma sodium mEq per litre	64	132 8 ± 1 2	10 0	131 — 141	flame photometry (Eppendorf)
Plasma potassium mEq per litre	64	4 85 ± 0 18	1 5	3 5 — 4 4	flame photometry (Eppendorf)
Serum chloride mEq per litre	62	93 9 ± 1 3	10 3	97 — 109	according to Scribner (1950)
Standard bicarbonate mEq per litre	60	18 7 ± 0 9	7 4	21 3 — 24 8	according to Andersen et al (1960)
Base excess mEq per litre	42	—6 7 ± 1 5	9 8	—2 3 — +2 3	according to Andersen et al (1960)
Serum calcium mEq per litre	51	4 06 ± 0 16	1 2	3 9 — 1 9	flame photometry (Eppendorf)
Inorganic serum phosphorus mg per cent	57	8 5 ± 0 6	4 3	2 5 — 4 7	according to Gomori (1942)
Serum magnesium mEq per litre	24	2 19 ± 0 16	0 8	1 3 — 2 3	according to Scharter (1961)
Serum protein g per cent	59	6 04 ± 0 13	1 0	6 8 — 8 2	biuret method

Compared with other method errors this may be considered as small In the following calculations of correlation coefficients etc, it was changes in the electrolyte concentrations that were taken into account For practical reasons the chloride concentrations in serum were used for the calculations instead of those in the extracellular fluid Similar considerations were also applicable to the bicarbonate concentration (Conway and Fearon 1944)

Nor was any correction factor used for the plasma or serum concentrations of the cations in the statistical calculations The correction for plasma water and the Donnan factor affect, for example the sodium concentration in different directions and they may be calculated approximately to equal each other Therefore the sodium concentration in the extracellular fluid can be assumed to be the same as that in plasma (Bergstrom, 1962)

C TREATMENTS WITH THE ARTIFICIAL KIDNEY

On 19 occasions an examination was carried out before and after a treatment with the artificial kidney The treatments were carried out with an apparatus designed according to Alwall (1963) with 20 metres of cellophane tubing giving a

Table 3 Electrolyte fluid balance and intramuscular temperature before and after treatments with the artificial kidney (dialysis and ultrafiltration)

	Number of treatments	Before the *treatments* Mean value ± S E	Mean difference between the *values before* and *after the* treatments ± S E	P
Non protein nitrogen mg per cent	19	203 ± 14	—89 ± 11	<0 001
Sodium mEq per litre	18	129 3 ± 2 6	—1 5 ± 1 4	—
Potassium mEq per litre	18	4 9 ± 0 3	—1 1 ± 0 25	<0 001
Chloride mEq per litre	19	90 5 ± 2 3	+2 7 ± 1 6	—
Standard bicarbonate mEq per litre	12	14 8 ± 1 8	+6 2 ± 1 2	<0 001
Calcium mEq per litre	7	4 4 ± 0 4	+0 4 ± 0 4	—
Inorganic phosphorus mg per cent	17	10 2 ± 0 9	—3 8 ± 0 6	<0 001
Magnesium mEq per litre	12	2 8 ± 0 3	—0 65 ± 0 25	<0 05
Body weight kilogram	16	65 3 ± 2 0	—1 60 ± 0 25[1])	<0 001
Temperature in musc biceps brachii °C	5	35 5 ± 0 2	+0 7 ± 0 5	—

[1]) In 13 patients expressed as the change in body weight during a 24 hour period

dialysing surface of about 16 000 sq cm With this apparatus it is possible in addition to dialysis to also produce an ultra filtration up to 1 litre per hour The average period of treatment was 6 5 hours (range 3 5 to 9 hours) The changes in the electrolyte fluid balance during the 19 treatments are shown in table 3 The concentrations of non protein nitrogen plasma potassium inorganic serum phosphorus and serum magnesium decreased significantly, while that of standard bicarbonate increased The concentrations of plasma sodium, serum chloride and calcium remained unchanged during the treatments The body weight was checked before the treatment The patient was weighed again after the treatment in 3 cases and on the following morning in 13 cases i e about 24 hours after the first weighing Even if this later weighing does not strictly fulfil the conditions for a post treatment weight most of the difference between this weight and the weight before treatment probably expresses the change in body weight occurring during the treatment with the artificial kidney On average 16 patients had lost 1 6 kg according to this calculation In 5 cases the temperature in the brachial biceps muscle was measured before and after the treatment with the artificial kidney and did not show any significant change

III METHODS

A ELECTROMYOGRAPHY

The electromyographic examination was carried out in the brachial biceps muscle The muscle action potentials were recorded photographically with a three channel electromyograph (DISA type 13 A 69) which had a frequency band of 2—10 000 c p s defined by 3 db discrimination an input impedance of 100 megohms shunted by 50 pF and a noise level of 3 μV r m s Concentric needle electrodes (DISA 13 K 03) were used with a leading off area of about 0 04 sq mm and an impedance of 50 000/—45° ohm at 150 c p s and 7 000/—41° ohm at 5 000 c p s The following electromyographic characteristics were studied (Buchthal 1957)

Mean duration of motor unit potentials

The mean duration of at least 20 different motor unit potentials (fig 2) was measured and the motor unit potentials were recorded at such a weak voluntary effort that the individual motor unit potentials could be delineated Three concentric needle electrodes were used simultaneously and each needle was inserted three times The distances between the needles and between the insertions were about 1 cm At each insertion recordings were made at three or more different

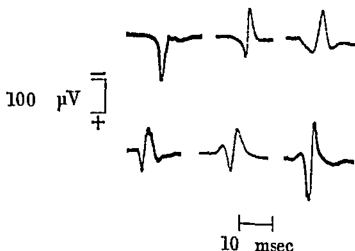


Fig 2 Motor unit potentials with 1—4 phases

depths in the muscle The recording was made at a sweep speed of 1 mm per msec and with an amplification giving 10 μV per mm Only motor unit potentials with an amplitude of 50 μV or more were considered

The amplification used permitted the initial and terminal components of the potentials to be measured with sufficient accuracy On the other hand it did not always permit measurement of the total amplitude of the individual action potentials The amplification was not reduced during the electromyographic examinations, which would have been necessary to measure the amplitudes of all the motor unit potentials adequately Thus no processing of the motor unit potential amplitude was carried out The duration of each separate motor unit potential was measured as the time interval between the first and last deviation from the base line (fig 3) The mean duration of motor unit potentials for the brachial biceps muscle varies with age so that it is prolonged with increasing age (Buchthal et al 1954 b) In this study normal values of mean duration of motor unit potentials at different ages according to data collected at the Laboratory of Clinical Neurophysiology, University Hospital, Copenhagen from 238 subjects (table 4), on 21 of whom the author carried out the examination to check the measurement technique were used The results were expressed in percentage deviation from the normal value for the age of the patient in question With one isolated examination the mean error of the mean duration of about 20—40 motor unit potentials is 5—7 per cent (Buchthal et al, 1954 a) The standard deviation for the mean duration of motor unit potentials for different individuals in the same age group is 10 per cent (Buchthal et al, 1954 b) and for the examination of a single patient only a deviation of more than 20 per cent from the normal value can be considered as pathological

Table 4 Normal values of mean duration of motor unit potentials in musc biceps brachii at different ages (238 subjects) according to data collected at the Laboratory of Clinical Neurophysiology University Hospital Copenhagen Normal range ± 20 per cent

Age in years	Mean duration of motor unit potentials in msec
15	9 6
18	9 8
20	10 0
25	10 3
30	10 6
35	10 9
40	11 1
45	11 2
50	11 4
55	11 6
60	11 9
65	12 2
70	12 4
75	12 6
80	12 8

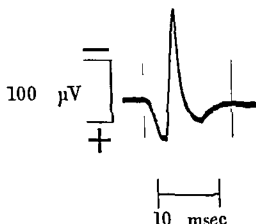


Fig 3 Triphasic motor unit potential showing starting and terminal points

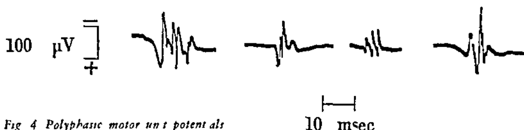


Fig 4 Polyphasic motor unit potentials

Polyphasic motor unit potentials

In conjunction with the measurements of the mean duration of motor unit potentials in the brachial biceps muscle those which were polyphasic were noted i e those with more than four phases (fig 4) The number of polyphasic potentials was expressed as a percentage of the number of potentials measured at each examination An incidence of 12 per cent polyphasic motor unit potentials formed the upper limit of the normal range (Caruso and Buchthal 1965)

Spontaneous activity

Spontaneous activity was recorded disregarding activity in the end plate zone such as end plate noise spontaneous negative discharges and spontaneous diphasic potentials with an initially negative phase (*Buchthal and Rosenfalck 1966*) and fibrillation potentials arising together with them In patients without signs or symptoms of neuromuscular disorders there were spontaneous fibrillation potentials in one place in 7 of 197 brachial biceps muscles (3 6 per cent) (Buchthal and Rosenfalck 1966)

Maximal voluntary effort

At maximal effort it was determined whether an interference pattern occurred or whether the pattern was sparser (mixed pattern and pattern of single motor unit potentials) (fig 5) The voltage

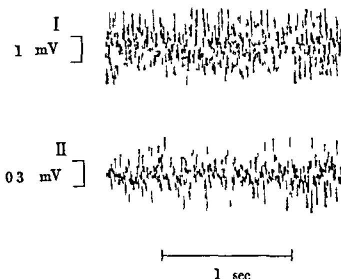


Fig 5 Action potential pattern at maximal voluntary contraction
I Interference pattern II Mixed pattern

of the pattern of motor unit potentials at maximal effort was measured between two lines drawn through the peaks in the recorded potentials but individual potentials were disregarded if they had an amplitude exceeding that of the majority In a normal brachial biceps muscle with good cooperation from the patient at maximal effort an interference pattern and an amplitude of about 3 mV are obtained However, the individual variation is great and only when the amplitude does not reach 1 5 mV can it be considered as reduced In gauging the results at maximal effort those with an interference pattern and amplitude of 1 5 mV or more were considered as normal and the remainder as abnormal

B MOTOR NERVE STIMULATION

The ulnar nerve was stimulated in the same arm in which the brachial biceps muscle was examined with electromyography Before stimulation the skin was scratched at the stimulation points with a fine grained sand paper and washed with ether in order to reduce the resistance of the skin during the stimulation The stimulus was rectangular, 0 7 msec in duration and applied via a double shielded transformer (DISA Multistim) to a bipolar surface electrode (electrode diameter 8 mm, electrode centre to centre separation 23 mm) successively placed along the nerve at the wrist and the elbow with the cathode distally placed The muscle action potentials in the abductor digiti quinti muscle were recorded with 1—3 concentric electrodes (DISA 13 K 51) (Trojaborg 1964) The strength of the stimulus was double the threshold value which was determined by an amplification of 10 μV per mm, but with a maximum of 50 volts, which was the highest stimulation voltage that the apparatus could provide During recording which was done with a sweep speed of 1 mm per msec the amplification was adjusted to a suitable magnitude The following parameters were studied (fig 6)

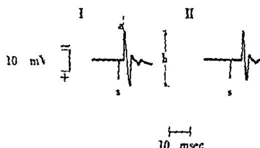


Fig 6 Action potential in the abductor digiti quinti muscle under stimulation of the ulnar nerve (I) at the wrist and (II) elbow a= distal latency b=peak to peak amplitude s= stimulation

Motor nerve conduction velocity

The motor nerve conduction velocity was measured in the ulnar nerve in the segment between the elbow (sulcus nervi ulnaris) and the wrist The distance between the two stimulation points was measured in whole cm and the difference between the latencies in the muscle action potential for the respective stimulation points in tenths of msec The conduction velocity was calculated with the aid of these magnitudes and expressed in m per sec If there were two or more simultaneous measurements, the result was given as their mean value In normal subjects the ulnar motor nerve conduction

velocity in the forearm in different studies was on average 56 to 59 m per sec with a S D of 5 m per sec (Henriksen 1956 Thomas et al 1959, Mayer, 1963 Trojaborg 1964)

Distal latency

The distal latency was measured as the time lag between the beginning of the stimulation and the initial deviation of the muscle action potential from the base line The channel in which the muscle action potential first had a negative phase was chosen for these measurements The distance between the distally placed cathode of the stimulation electrode and the recording electrode was measured to the nearest 0 5 cm and was on the average 6 5 (4—9) cm To make the measured distal latencies as comparable as possible they were corrected with regard to the distance to a point lying 6 5 cm from the recording electrode This latency was called the corrected delay and was obtained according to the formula

$$\text{corrected delay (msec)} = \text{measured delay (msec)} + \frac{(6\,5 - \text{measured distance in cm}) \times 10}{\text{motor nerve conduction velocity (m/sec)}}$$

in which the motor nerve conduction velocity was used as measured for the ulnar nerve in the forearm in each patient Carpendale (1956) found the distal conduction time on stimulation of the ulnar nerve at the wrist and recording of the action potential in the abductor digiti quinti muscle in 30 normal subjects to be on average 2 7 msec (range 2 1 to 3 4 msec, distance 6 4 cm) and Mavor and Libman (1962) on examination of 7 subjects found it to be 2 8 msec (range 2 3 to 3 4 msec)

Amplitude of the muscle action potential

The peak to peak amplitude was measured for the muscle action potentials recorded in the abductor digiti quinti muscle on stimulation of the ulnar nerve at the wrist The channel in which the muscle action potential had the highest amplitude was chosen for these measurements This method of measuring the amplitudes of muscle action potentials with intramuscular concentric needle electrodes is impaired by a great degree of inaccuracy since among other things varying needle positions can give great variations in the results Therefore only large changes of amplitude can permit any conclusions Trojaborg (1964) found the corresponding amplitude in 30 normal subjects to be 16 8±0 8 mV (S D 4 7 mV)

The distal latencies and amplitudes in the muscle action potentials did not depend on the stimulation strength as long as this was at least 1 5 times the threshold value Therefore 10 patients were included in the material where the stimulation strength could not attain double the threshold but at least 1 5 times that value In examinations with stimulation strengths less than 50 per cent higher than the threshold value a distal latency prolonged compared with the rest and a reduced amplitude in the muscle action potentials were observed These were excluded from the examinations

C REPETITIVE MOTOR NERVE STIMULATION

Repetitive stimuli were given to the ulnar nerve by means of two needle electrodes (electrode diameter 0 5 mm elec

trode distance 10—20 mm) inserted in the proximity of the nerve at the wrist The supramaximal stimulus (at least twice threshold) was rectangular 0 7 msec in duration and applied via a double shielded transformer (DISA Multistim) The duration of the repetitive stimulation was 1 sec and the frequencies were 10 30 and 50 per sec The recording of the stimulation response in the abductor digiti quinti muscle was done by means of concentric needle electrodes (DISA 13 K 51) During this part of the examination the frequency band of the electromyograph (DISA type 13 A 69) was 20—10 000 c p s During the examination the hand and forearm were fastened in an arm splint to reduce the disturbances caused by movement

An isolated stimulation was given after each repetitive stimulation On each occasion the stimulator had to be changed manually from train to single stimulation therefore the time interval between the end of the repetitive stimulation and the isolated stimulation varied and was on average 1 12±0 01 sec (S D 0 20 sec) The following parameters were studied (fig 7)

Change in amplitude in the muscle action potential under repetitive nerve stimulation

In the case of the repetitive stimulation the amplitudes in the first and last muscle action potential were compared The results were expressed as the percentage increase or decrease in the amplitude of the last potential compared with the first With repetitive stimulation of the ulnar nerve in normal subjects with frequencies up to 40 per sec Harvey and Masland (1941) found no essential changes of amplitude in the action potential in the abductor digiti quinti muscle during a 3 second period With frequencies between 40 and 60 per sec there was a gradual decrease in the amplitudes from 5 to 15 per cent during the same period

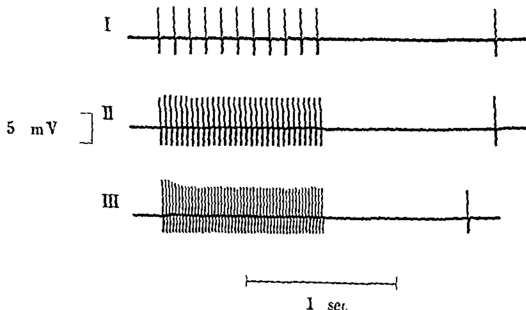


Fig 7 Muscle action potentials under repetitive motor nerve stimulation at the frequencies of 10 (I) 30 (II) and 50 (III) per sec and under post tetanic motor nerve stimulation

Change in amplitude in the muscle action potential with isolated nerve stimulation after a repetitive stimulation

During the post tetanic stimulation the amplitude in the muscle action potential was measured and the result expressed as the percentage change in relation to the amplitude in the first potential during the previous repetitive stimulation In normal subjects the amplitude in the muscle action potential during isolated nerve stimulation after a tetanic stimulation with corresponding frequencies was not significantly changed (Botelho and Cander 1953, Grob et al 1957)

In cases where two or three simultaneous recordings were made the mean value of the results obtained was used for the calculations

D INTRAMUSCULAR TEMPERATURE

Intramuscular temperatures were recorded with an Electric Universal Thermometer type TE 3 (Ellab Copenhagen) The temperatures were measured with an accuracy of 0 1 °C An electrode with a thermo junction shaped like an ordinary injection needle (type K 8 diameter 0 7 mm length 50 mm) was used as an applicator The intramuscular temperature was measured in the brachial biceps muscle examined by electromyography The thermo needle was inserted approximately in the centre of the area examined

E MUSCLE BIOPSY

The muscle biopsies were taken from the anterior tibial muscle with two nasal forceps according to Weil Blakesley size no 3 with a semi micro technique (Radner 1965) One or two pieces of muscle were taken with one pair of forceps for histological examination which was carried out after fixing in Bouin s solution and staining with hematoxylin and eosin and according to Hansen van Gieson A piece of muscle was taken with the other pair of forceps for analysis of the water content After being rolled on a plastic surface thus removing a quantity of blood the piece of muscle was placed in a small glass bowl which had been weighed beforehand A first weighing of the muscle was made 1—3 min after it had been extracted and since its weight reduction by evaporation during the first minutes was linear it was possible to calculate the original weight by means of weight data for each minute up to 11—12 min (Bergstrom 1962) The muscle was then dried in an oven at 95—100 °C for at least 24 hours after which it was then re weighed and the water content calculated At this temperature the pieces of muscle reached constant weight after a few hours The muscle was then placed in 25 ml re distilled petroleum ether (boiling point 30—60 °C) for 24 hours after which it was re weighed after further drying for at least three hours This weighing gave the quantity of fat free solids in the muscle The total quantity of water in the muscle (H_2O_m) was expressed in grammes of total water per 100 grammes of fat free solids (FFS)

F STATISTICAL METHODS

The following formulae were used for the statistical calculations (Snedecor 1956 Kemp and Nielsen 1961, Rao 1962)

The mean value $\bar{x} = \frac{\sum x_i}{n}$, where x_i is the individual observations and n the number of observations

The standard deviation (S D)

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

The standard error of the mean (S E)

$$s_{\bar{x}} = \frac{s}{\sqrt{n}}$$

The following signs are used

$$S_{xx} = \sum(x_i - \bar{x})^2 = \sum x_i^2 - \frac{(\sum x_i)^2}{n}$$

$$S_{xy} = \sum(x_i - \bar{x})(y_i - \bar{y}) = \sum x_i y_i - \frac{(\sum x_i)(\sum y_i)}{n},$$

$$S_{yy} = \sum(y_i - \bar{y})^2 = \sum y_i - \frac{(\sum y_i)^2}{n} \quad \text{etc}$$

To determine whether two mean values $\bar{x}_1$ of n_1 observations and $\bar{x}$ of n_2 observations differed significantly the t test was used

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{S_{x_1x_1} + S_{x_2x_2}}{n_1 + n - 2}\left(\frac{1}{n_1} + \frac{1}{n}\right)}}$$

where the significance limits for t were evaluated in tables (Documenta Geigy Wissenschaftliche Tabellen) with $n_1 + n_2 - 2$ degrees of freedom

Where the p values were less than 0 05 the differences were considered significant

With the treatment of pairs of observations the differences d between the coupled observations gave the mean value

$$\bar{d} = \frac{\sum d}{n} \quad \text{and} \quad t = \frac{\bar{d}}{s_{\bar{d}}}$$

where t had n — 1 degrees of freedom

The correlation coefficient r between two variables x and y was calculated according to the formula

$$r = \frac{S_{xy}}{\sqrt{S_{xx} \; S_{yy}}}$$

The significance for r was tested with

$$t = \frac{r}{s_r}$$

where t had n — 2 degrees of freedom and

$$s_r = \sqrt{\frac{1 - r^2}{n - 2}}$$

For the regression line $y = bx + a$, where x usually indicated a blood factor and y an electromyographic or corresponding factor, the constants b were estimated with

$$b^* = \frac{S_{xy}}{S_{xx}} \quad \text{and a with} \quad a^* = \bar{y} - b^*\bar{x}$$

To determine whether the regression lines for two random tests $y = bx + a$ and $y = b\ x + a$ differed significantly,

$$s^2 = \frac{(n-2)\, s^2 + (n'-2)\, s^2}{n+n'-4}$$

was used where

$$s = \frac{1}{n-2}\left(S_{yy} - \frac{S^2_{xy}}{S_{xx}}\right) \text{ and}$$

$$s^2 = \frac{1}{n-2}\left(S_{yy} - \frac{S^2_{xy}}{S_{xx}}\right),$$

the regression coefficients were then tested according to the formula

$$t = \frac{b^* - b'^*}{s\sqrt{\frac{1}{S_{xx}} + \frac{1}{S_{xx}}}}$$

where t had n+n — 4 degrees of free dom

If b and b did not differ significantly a and a were tested according to the for mula

$$t = \frac{a^* - a^*}{s\sqrt{\frac{1}{n} + \frac{1}{n} - \frac{(\bar{x})^2}{S_{xx}} + \frac{(\bar{x}')^2}{S_{xx}}}}$$

where t had n+n — 4 degrees of freedom

In calculating partial correlation coef ficients between the variables x y and z, r_{xy}, r_{xz} and r_{yz} were calculated first The partial correlation coefficient r_{xz} with the effect of y eliminated was

$$r_{xz\,y} = \frac{r_{xz} - r_{xy}\ \ r_{yz}}{\sqrt{(1-r_{xy})\ (1-r_{yz})}}$$

The significance was tested with

$$t = \frac{r_{xz\,y}}{\sqrt{(1-r_{xz\,y})}}\ \sqrt{n-3}$$

where t had n — 3 degrees of freedom and n was the lowest n for r_{xy}, r_{xz} or r_{yz}

Similarly

$$r_{yz\,x} = \frac{r_{yz} - r_{xy}\ \ r_{xz}}{\sqrt{(1-r^2_{xy})\ (1-r^2_{xz})}}$$

For a multiple regression equation with the measured variables x_1 x_2 and y the constants in the equation $y = b_1x_1 + b_2x_2 + a$ were estimated with

$$b^*_1 = \frac{S_{x_2x_2}\ \ S_{yx_1} - S_{x_1x_2}\ \ S_{yx_2}}{S_{x_1x_1}\ \ S_{x_2x_2} - (S_{x_1x_2})^2}$$

$$b^*_2 = \frac{S_{x_1x_1}\ \ S_{yx_2} - S_{x_1x_2}\ \ S_{yx_1}}{S_{x_1x_1}\ \ S_{x_2x_2} - (S_{x_1x_2})^2}$$

$$a^* = \bar{y} - b^*_1\bar{x}_1 - b^*_2\bar{x}_2$$

The coefficient of multiple correlation R was obtained by means of

$$R = \frac{S_{y\hat{y}}}{\sqrt{S_{yy}\ \ S_{\hat{y}\hat{y}}}}$$

where $\hat{y}$ was the values in the obser vation y anticipated by means of the equation

To compare two frequencies $\frac{x_1}{n_1}$ and $\frac{x_2}{n_2}$

the χ^2 analysis was used according to the

following formula

$$\chi^2 = \frac{(n_1+n_2)\left(\left|x_1(n_2-x_2)-x_2(n_1-x_1)\right|-\frac{n_1+n_2}{2}\right)^2}{n_1\,n_2\,(x_1+x_2)(n_1+n_2-x_1-x_2)}$$

with 1 degree of freedom

IV RESULTS

In the following results the individual parameters for electromyography and motor nerve stimulation are considered first and their relation to uraemia and electrolyte concentrations analysed If nothing to the contrary is indicated in calculating mean values correlation coefficients etc only one examination is included for each patient and this examination was not carried out immediately after a treatment with the artificial kidney Finally the results of examinations carried out before and after a treatment with the artificial kidney are reported The figures for each individual examination are tabulated in the appendix

A MEAN DURATION OF MOTOR UNIT POTENTIALS

In 64 of the 65 patients included in the study the mean duration of motor unit potentials was on the average + 2 4 ± 1 3 per cent (S D 10 5 per cent) compared with the normal value for each patient s age group In the case of one patient the mean duration of motor unit potentials was not determined owing to a profuse spontaneous activity The mean duration of motor unit potentials of one patient was reduced by more than 20 per cent of the normal value and was 8 3 msec (EMG 62)

The patient was a 65 year old woman with a chronic glomerulonephritis with a tendency to nephrosis a high degree of uraemia and metabolic acidosis Simultaneous electrolyte determinations were lacking but a day before the examination the concentration of plasma sodium was 148 mEq per litre and plasma potassium 5 3 mEq per litre The incidence of polyphasic motor unit potentials was 9 per cent No muscle biopsy or motor nerve stimulation was carried out in this case

The mean duration of motor potentials in three patients was increased by more than 20 per cent of the normal value and was 13 2 msec (26 years of age) (EMG 566), 11 8 msec (15 years of age) (EMG 207) and 12 6 msec (24 years of age) (EMG 38) respectively

The incidence of polyphasic motor unit potentials was 0—2 per cent in all cases which did not otherwise show any common features The diagnoses of these three patients were chronic interstitial nephritis acute renal failure (tubular necrosis) after suspected hypersensitivity to medicine and gastritis with prerenal electrolyte disturbances In the latter case there was a hypernatremia (168 mEq per litre) and hypopotassemia (1 6 mEq per litre) while the two other patients had hyponatremia (122 and 117 mEq per litre) and hyperpotassemia (5 4 and 6 2 mEq per litre) The patient with the interstitial nephritis had a prolonged uraemia the motor nerve conduction velocity was reduced and the distal latency increased The patient with the acute renal failure had a normal motor nerve conduction velocity and distal latency as had also the patient with the gastritis at a later examination

There was no significant linear correlation between the mean duration of motor

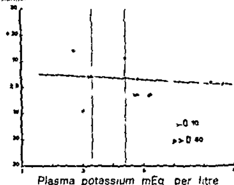


Fig 8 The relation between the mean duration of motor unit potentials and plasma potassium concentration The equation for the regression line is y=—0 69 x+6 31
In this and the following figures the normal range among the different blood factors is marked by thin vertical lines

unit potentials and the plasma potassium concentration (fig 8) or other examined blood factors or the intramuscular temperature A more detailed analysis revealed however that the mean duration of motor unit potentials was prolonged in cases with hyponatremia, which is shown in table 5 and fig 9 In the group with the plasma sodium concentration less than 131 mEq per litre the percentage deviation of the mean duration of motor unit potentials was +7 0±2 1 per cent, which differed from the corresponding value of —0 4±1 4 per cent for the group with the plasma sodium concentration 131—141 mEq per litre (p<0 005) For the examinations with the plasma sodium concentration 141 mEq per litre or lower

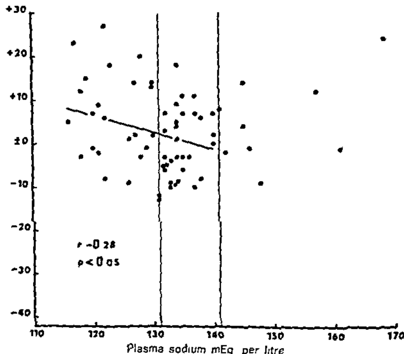


Fig 9 The correlation between the mean duration of motor unit potentials and plasma sodium concentration Examinations with plasma sodium concentration more than 141 mEq per litre were not included in the calculation of the regression line the equation of which is y=—0 40 x+54 1

Table 5 Percentage deviation of the mean durations of motor unit potentials divided into groups in relation to the concentration of plasma sodium

Plasma sodium mEq per litre	Number of observations	Plasma sodium mEq per litre Mean value	Mean duration of motor unit potentials Per cent deviation from normal ± S E
< 131	23	123 5	+7 0 ± 2 1
131 — 141	33	134 8	—0 4 ± 1 4
> 141	8	151 5	+5 1 ± 3 8

there was a significant linear correlation ($p < 0\ 05$) between the plasma sodium concentration and the mean duration of motor unit potentials which fell by 0 4 per cent for each increase of 1 mEq per litre in the plasma sodium concentration

In fig 10, 26 patients with two examinations are arranged with regard

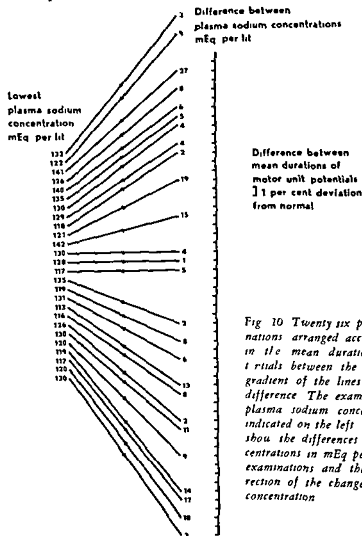


Fig 10 Twenty six patients with two examinations arranged according to the difference in the mean duration of motor unit potentials between the two examinations The gradient of the lines is proportional to this difference The examination with the lowest plasma sodium concentration is placed and indicated on the left The figures on the right show the differences in plasma sodium concentrations in mEq per litre between the two examinations and the arrows show the direction of the change in the plasma sodium concentration

to the difference in the mean durations of motor unit potentials between the two examinations The examination with the lowest plasma sodium concentration is placed on the left. The figures on the right show the difference in the plasma sodium concentrations in mEq per litre between the two examinations and the arrows show the direction of the change in the plasma sodium concentration The gradient of the lines is proportional to the change in the mean duration of motor unit potentials An upward gradient indicates an increase and a downward slope a decrease There was an increase in 11 cases and a decrease in 12 Table 6 shows that the change in the plasma sodium concentration was the same in the group with the increasing duration of the potentials as that in the group with the decreasing duration and similarly that the magnitude of the change in the mean duration of motor unit potentials was the same in both groups The lowest plasma sodium concentration for the group with the decreasing potential duration was on the average 123 0±2 0 mEq per litre and for the group with increasing potential duration 130 5±2 5 mEq per litre, between these concentrations the difference was significant ($p<0\ 05$) This was compatible with what was shown above, that is that the mean duration of motor unit potentials was prolonged with hyponatremia, a plasma sodium concentration rising from a low to a higher value caused a decreasing mean duration of motor unit potentials In addition the difference between the two groups may be compatible with the fact that the mean duration of motor unit potentials increased if the plasma sodium concentration further increased from a high value, which would indicate a minimum in the function by which the mean duration of motor unit potentials was dependent on the plasma sodium concentration If the correlation between the mean durations of motor unit potentials and plasma sodium concentrations was calculated for the examinations with a plasma sodium concentration of 131 mEq per litre or higher (cf fig 9) there was a significant positive correlation with the correlation coefficient $r=0\ 44$ ($p<0\ 005$) However if one single ex

Table 6 Twenty three patients with two examinations grouped with the examination with the lowest plasma sodium concentration as starting point in relation to whether the mean duration of motor unit potentials increased or decreased

Mean duration of motor unit potentials	Increasing	Decreasing	p
Number of patients	11	12	
	Mean value±S E	Mean value±S E	
Lowest plasma sodium concentration, mEq per litre	130 5±2 5	123 0±2 0	<0 05
Mean difference between plasma sodium concentrations mEq per litre	9 3±2 4	9 3±1 6	—
Mean difference between mean durations of motor unit potentials per cent deviation from normal	+9 3±1 0	—11 5±1 3	—

treme value with the highest plasma sodium concentration was excluded in the calculations the correlation was no longer significant (r = 0 25, p > 0 10)

Another method of evaluating these 26 patients examined twice was to correlate the change in the plasma sodium concentration with the change in the mean duration of motor unit potentials In 23 patients with two examinations with the plasma sodium concentration 141 mEq per litre or lower this correlation was significant with the correlation coefficient r = —0 44 (p < 0 05) while the correlation coefficient was r = 0 51 (p > 0 20) in 7 patients with two examinations with the plasma sodium concentrations 131 mEq per litre or higher

In patients with low plasma sodium concentrations the mean duration of motor unit potentials was therefore prolonged The number of observations with high plasma sodium concentrations was small and did not permit any conclusions but the results indicate that the mean duration of motor unit potentials could also be prolonged in the case of hypernatremia

B POLYPHASIC MOTOR UNIT POTENTIALS

In 64 of the 65 patients covered by the study the incidence of polyphasic motor unit potentials was on the average 2 7 ± 0 4 per cent (S D 3 4 per cent) In one case the incidence of polyphasic motor unit potentials could not be determined owing to profuse spontaneous activity More than 12 per cent of polyphasic potentials was present in only one case who had hypercalcemia (6 4 mEq per litre) and a moderate alkalosis

The patient was a 62 year old man with an anamnesis with a tendency to alcoholism and a slight untreated diabetes mellitus He was sent to the clinic for dialysis treatment for hypercalcemia He was listless and not quite lucid The knee and heel reflexes were absent Under electromyography there was no spontaneous activity the pattern at maximal effort the mean duration of motor unit potentials and corrected delay were normal The ulnar nerve conduction velocity was low (42 m per sec) The patient died later and the autopsy revealed a primary cancer of the liver with metastases

As an explanation of the low motor nerve conduction velocity there was not only a moderate uraemia (Chap IV E) but also the patient s malignity (Croft and Wilkinson 1965) diabetes mellitus (Mulder et al 1961 Skillman et al 1961) and alcoholic tendency (Ramelli and Zerbi 1962 Mawdsley and Mayer 1965)

The incidence of polyphasic motor unit potentials was significantly correlated with the concentration of serum calcium and standard bicarbonate and base excess but not with the concentration of any of the other electrolytes non protein nitrogen or intramuscular temperature With increasing serum calcium concentration the incidence of polyphasic motor unit potentials rose by 1 27 per cent per mEq per litre (p < 0 005) (fig 11) However, this significance depended on too few cases of hypercalcemia and disappeared if the three examinations with the highest serum calcium concentrations were eliminated from the calculations

These three examinations concerned the patient mentioned above and the two patients with hyperparathyroidism included in the study In neither of the latter cases were there any criteria for neuropathy neither had uraemia, the reflexes were normal, the ulnar nerve conduction velocity was normal (55 and 64 m per sec) and the corrected delay was determined in one of the cases as 2 4 msec

The incidence of polyphasic motor unit potentials also rose with increasing stan

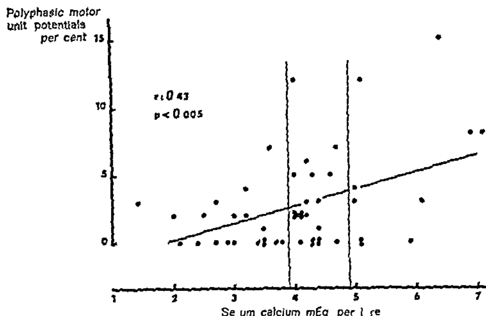


Fig 11 The relat on between the inc dence of polyphas c motor un t potentials and the con central on of serum calc um The eq at on for the regress on l ne is y=1 27 x—2 40

dard bicarbonate (fig 12) and base excess Both standard bicarbonate (fig 13) and base excess however showed a co variation with serum calcium and if this was taken into consideration through the calculation of partial correlation coefficients the significance for these correlations disappeared (see table 7)

C SPONTANEOUS ACTIVITY

A profuse spontaneous activity of short potentials (mean duration 4 msec) was registered in one case (EMG 529) These potentials differed from fibrillation potentials inasmuch as many were of complex form The patient was a non uraemic

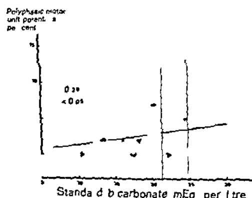


Fig 12 The relat on between the inc dence of polyphas c motor un t potent als and the con central on of standard b carbonate The equa tion for the regre s on l ne is y 0 16 x — 0 25

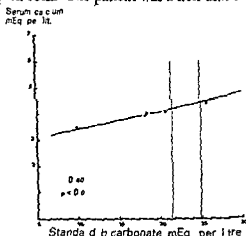


Fig 13 The relat or between the concen trat on of serum calcium and standard bicar bonate The equat o for the regression l ne y 0 07 x + 2 72

Table 7 The correlation coefficients for the relations between the incidence of polyphasic motor unit potentials and the concentrations of serum calcium standard bicarbonate and base excess

	Correlation coefficient		Partial correlation coefficient — calcium effect eliminated	
Calcium				
— polyphasic potentials	0 42	($p < 0\ 005$)	—	
Standard bicarbonate				
— polyphasic potentials	0 29	($p < 0\ 05$)	0 15	($p > 0\ 30$)
Base excess				
— polyphasic potentials	0 34	($p < 0\ 025$)	0 16	($p > 0\ 30$)

woman who owing to extreme vomiting for a long period had a hypochloremic alkalosis and a hypopotassemia and hypo magnesemia

Among the 109 examinations included in the study spontaneous fibrillation po tentials with a duration of 2 5 to 6 5 msec (fig 14) occurred in 10 cases (9 2 per cent) Eight patients had fibrillation po tentials in one point in the muscle only Fibrillation potentials at two points in the muscle were registered in two patients One was the patient mentioned above with a profuse spontaneous activity at a second examination when the electrolyte balance was almost normalised The other patient was also non uraemic and had a hypopotassemia and metabolic alkalosis

The non protein nitrogen and electrolyte concentrations for the ten patients with spontaneous fibrillation potentials are summarised in table 8 where they are compared with the corresponding values for the other examinations in which there were no fibrillation potentials There was a difference between the groups in the concentrations of plasma potassium which was 25 per cent lower for the group with fibrillation potentials compared with the group without them ($p<0\ 01$) In addi tion the group with fibrillation potentials had a concentration of plasma sodium 6 per cent higher ($p<0\ 01$) and a serum chloride concentration 11 per cent higher ($p<0\ 005$) than the group without fibril lation potentials In table 9 all the exami

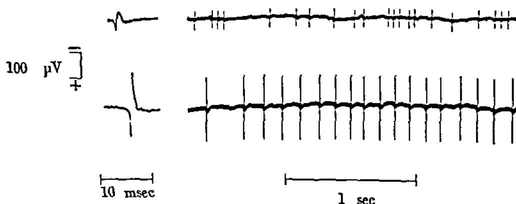


Fig 14 Spontaneous fibrillation potentials *(Slightly retouched)*

Table 8 Non protein nitrogen and electrolyte concentrations for examinations with and without spontaneous fibrillation potentials

Blood component	With fibrillation potentials Number	With fibrillation potentials Mean value ± S E	Without fibrillation potentials Number	Without fibrillation potentials Mean value ± S E	P
Non protein nitrogen mg per cent	8	125 ± 31	92	158 ± 8	—
Sodium mEq per litre	10	139 0 ± 2 8	94	130 6 ± 0 9	< 0 01
Potassium mEq per litre	10	3 46 ± 0 3	95	4 64 ± 0 13	< 0 01
Chloride mEq per litre	9	103 3 ± 3 1	93	92 9 ± 1 0	< 0 005
Standard bicarbonate mEq per litre	9	24 0 ± 2 3	85	19 4 ± 0 7	—
Base excess mEq per litre	9	+0 3 ± 3 2	52	—5 6 ± 1 2	—
Calcium mEq per litre	6	5 1 ± 0 7	60	4 03 ± 0 14	—
Inorganic phosphorus mg per cent	6	6 1 ± 2 5	85	8 3 ± 0 4	—
Magnesium mEq per litre	2	2 5 (range 1 7 — 3 3)	41	2 29 ± 0 13	—

Talle 9 Incidence of spontaneous fibrillation potentials in groups of examinations divided according to the plasma potassium plasma sodium and serum chloride concentration respectively All examinations included

Blood component	Concentration mEq per litre	Number of examinations	With spontaneous fibrillation potentials Number	With spontaneous fibrillation potentials Percentage
Plasma potassium	< 3 3	16	4	25
	3 3 — 4 4	40	5	13
	> 4 4	49	1	2
Plasma sodium	< 131	46	0	0
	131 — 141	49	8	16
	> 141	9	2	22
Serum chloride	< 97	60	1	2
	97 — 108	37	7	19
	> 108	5	1	20

nations have been divided up into groups with regard to reduced normal and increased concentrations of plasma potassium and sodium and serum chloride The incidence of fibrillation potentials for the group with hypopotassemia was higher than that for the group with hyperpotassemia (p<0 005) There was no difference in the incidence between the hypopotassemic group and the group with normal plasma potassium concentration However if the examinations with the plasma potassium concentration 3 3 mEq per litre were transferred to the group with hypopotassemia this group covered 21 examinations 7 of which with spontaneous fibrillation potentials (33 per cent), while the 35 examinations in the remaining group with normal plasma potassium concentration included 2 with fibrillation potentials (6 per cent), the difference between these incidences was significant (p<0 025) There were no fibrillation potentials in the group with hyponatremia which distinguished it from the groups with normal and increased plasma sodium concentration (p<0 025) The incidence of fibrillation potentials was significantly lower in the hypochloremic group than in the group with normal serum chloride concentration (p<0 01) The serum chloride concentration showed a co variation with the plasma sodium concentration with the correlation coefficient r=0 56 (p<0 001) On the other hand the concentration of plasma potassium was not significantly correlated with that for plasma sodium or serum chloride

Spontaneous positive sharp waves (fig 15) with a duration of 3—12 msec occurred in 4 patients (EMG 152, 530, 552 and 566) These showed no common feature differing from the other patients in their uraemia electrolyte pattern

Pseudomyotonic bursts (fig 15) were recorded in 5 patients (EMG 120, 152 530 552 and 566) whose uraemia electrolyte condition did not differ significantly from that of the other patients

D MAXIMAL VOLUNTARY EFFORT

On maximal voluntary contraction 47 patients displayed an interference pattern with the amplitude 1 5 mV or more Of the remaining 18 patients 4 had an interference pattern with a lower amplitude, 1 a mixed pattern with the amplitude 1 6 mV and 13 a mixed pattern or a pattern of single motor unit potentials with an amplitude lower than 1 5 mV The plasma potassium concentration for the group of patients who were unable to display an interference pattern with the amplitude 1 5 mV or more was 36 per cent higher than that for the group who achieved this contraction pattern (p<0 001) (table 10) There were however also other significant differences between these two groups, the hyperpo

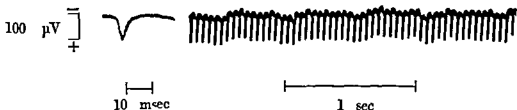


Fig 15 Spontaneous positive sharp waves in a pseudomyotonic burst

Table 10 Non protein nitrogen and electrolyte concentrations for the patients who at maximal voluntary contraction were able to show an interference pattern and amplitude 1 5 mV or more, compared with the corresponding values for the remaining patients with sparser activity patterns

Blood component	Interference pattern and amplitude ≥ 1 5 mV		Remaining patients		p
	Number	Mean value ± S E	Number	Mean value ± S E	
Non protein nitrogen mg per cent	42	156 ± 13	17	205 ± 18	<0 05
Sodium mEq per litre	47	132 1 ± 1 4	17	131 8 ± 2 7	—
Potassium mEq per litre	47	4 42 ± 0 18	17	6 02 ± 0 36	<0 001
Chloride mEq per litre	46	93 0 ± 1 5	16	96 3 ± 2 8	—
Standard bicarbonate mEq per litre	43	19 9 ± 1 1	17	15 6 ± 1 6	<0 05
Base excess mEq per litre	31	—4 5 ± 1 7	11	—13 0 ± 2 3	<0 02
Calcium mEq per litre	37	4 05 ± 0 17	14	4 08 ± 0 41	—
Inorganic phosphorus mg per cent	42	7 7 ± 0 6	15	10 8 ± 1 2	<0 02
Magnesium mEq per litre	19	2 10 ± 0 19	5	2 5 ± 0 2	—

tassemic group also had a higher degree of uraemia and hyperphosphatemia together with a more pronounced metabolic acidosis than the other group

To evaluate the activity pattern at maximal voluntary contraction accurately however good co-operation was required on the part of the patient Such was not always the case which could be attributed not only to uraemia and electrolyte disturbances but also to other circumstances such as a bad general condition due to general infections vomiting etc Thus the general condition of the 47 patients who could display an interference pattern with the amplitude 1 5 mV or more was classified as bad in 1 patient, while in the case of the 18 patients who did not achieve this contraction pattern the general condition was bad in 15 patients

To test whether the differences in the uraemia and electrolyte condition between the two groups caused a general difference in the contraction potential of the musculature the amplitudes of the action potential in the abductor digiti quinti muscle under stimulation of the ulnar nerve for both groups were compared This amplitude among the 32 patients in the group with the interference pattern and amplitude 1 5 mV or more at maximal voluntary contraction was on the average 18 5±1 1 mV and in the 9 patients in the other group on the average 14 5±2 0 mV thus there was no significant difference between these amplitudes

E MOTOR NERVE CONDUCTION VELOCITY

The conduction velocity in the ulnar nerve in the section between the elbow and the wrist was determined in 54 patients and was on the average 55 9 ± 1 0 m per sec (S D 7 6 m per sec) There were no statistically significant linear correlations between the motor nerve conduction velocity and the concentration of plasma sodium, serum chloride standard bicarbonate base excess calcium inorganic phosphorus or magnesium

Motor nerve conduction velocity and non protein nitrogen

The relation between the motor nerve conduction velocity and the degree of uraemia expressed as non protein nitrogen concentration is shown in fig 16 With increasing uraemia the nerve conduction velocity fell, the correlation coefficient being $r = -0\ 45$ ($p < 0\ 005$) An increase of 100 mg per cent non protein nitrogen meant a decrease of 4 0 m per sec in the motor nerve conduction velocity

Motor nerve conduction velocity and plasma potassium

There was a connection between the motor nerve conduction velocity and plasma potassium concentration with the correlation coefficient $r = -0\ 36$ ($p < 0\ 01$) (fig 17) An increase of 1 mEq per litre of plasma potassium meant a decrease of 2 1 m per sec in the motor nerve conduction velocity However, in these cases there was also a connection between non protein nitrogen and plasma potassium whereby the potassium concentration in

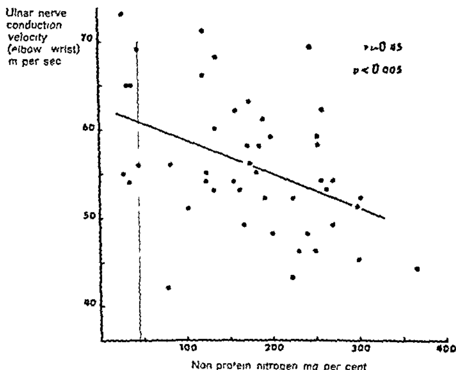
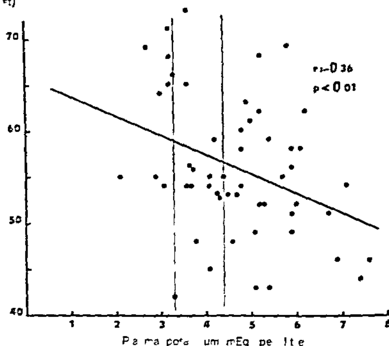


Fig 16 The relation between motor nerve conduction velocity and non protein nitrogen concentration The equation for the regression line is $y = -0\ 040\ x + 62\ 8$



T e e a n he ue n mo or nerve ondu t n velocity and plasma potass um concen
T q a n or the regre on l re y —2 1 x + 66 0

creased w th rising non protein nitrogen con entration, the correlation coefficient being r – 0 58 (p<0 001) (fig 18) To

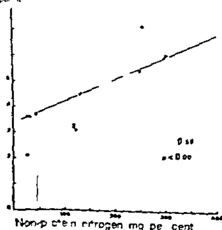


Fig 18 The relat on between the con en tra ons of non protein n trogen and pla ma potass um The equat on for the regre on l ne is y 0 0087 x + 3 32

ascertain which of the factors non protein nitrogen and plasma potassium was crucial for the motor nerve conduction velocity the material was divided into sub groups the first group having plasma potassium concentration 3 3 to 4 4 (mean alue 3 84) mEq per litre and the second group having plasma potassium concen tration ≥5 0 (mean value 5 94) mEq per litre In these sub-groups covering 14 and 22 cases respectively the regression lines for the dependence of the motor nerve conduction velocity on non protein nitrogen concentration were calculated Figure 19 shows that the regression lines for both groups had great similarity and that there were no statistically verified differences between them A correspond ing division of the material into sub-groups according to non protein nitrogen

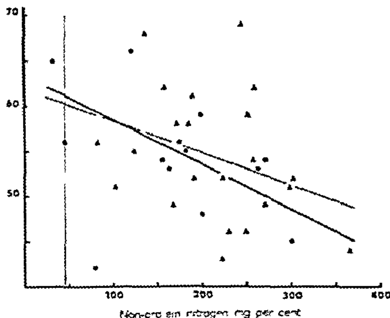


Fig 19 The relation between motor nerve conduction velocity and non protein nitrogen concentration for two groups with different plasma potassium concentration The group with the plasma potassium concentration 3 3 to 4 4 mEq per litre is marked with dots and a fully drawn regression line with the equation y = —0 0[illegible]9 x + 65 4 The group with the plasma potassium concentration 5 0 mEq per litre or more is marked with triangles and a broken regression line with the equation y = —0 035 x + 61 8

was of no evidential value, since in the group with normal concentrations of non protein nitrogen there was merely an increased potassium value Another method of evaluating the dependence of the motor nerve conduction velocity on the concentrations of non protein nitrogen and plasma potassium was to compare the mutual correlation coefficients of the three parameters If the partial correlation coefficient between the motor nerve conduction velocity and the non protein nitrogen concentration was obtained with the potassium effect eliminated it was r = —0 32 (p < 0 05) If on the other hand the influence of non protein nitrogen on the relation between the motor nerve conduction velocity and the plasma potassium concentration was eliminated the partial correlation coefficient was r = —0 14 (p > 0 30) Thus the plasma potassium concentration did not influence the motor nerve conduction velocity which was on the other hand dependent on the concentration of non protein nitrogen. The correlation observed between the motor nerve conduction velocity and the potassium concentration was merely dependent on the fact that the latter showed a co-variation with the concentration of non protein nitrogen.

Motor nerve conduction velocity and duration of uraemia

To ascertain whether the period during which the patients had had uraemia affected the motor nerve conduction velocity the patients were divided into three groups: those without uraemia (7 patients), those who had had uraemia anamnestic or verified by laboratory test for one month or less (22 patients), and those who had had such a uraemia for longer than one month (18 patients) (table 11). The group with uraemia for more than a month had a motor nerve conduction velocity 16 per cent lower than the group of non-uraemic patients ($p < 0.001$). There were no significant differences between the motor nerve conduction velocities in the other groups. The regression lines for the variation of the motor nerve conduction velocity with non-protein nitrogen concentration were plotted for the two uraemic groups (fig. 20), between which there was no significant difference.

F. DISTAL LATENCY

The distal latency of the action potential in the abductor digiti quinti muscle on stimulation of the ulnar nerve at the wrist in 48 patients was on the average 2.75 ± 0.07 msec (S.D. 0.46 msec). After correction to a fixed distance (6.5 cm) between the stimulation and recording electrodes in 47 patients the corrected delay was 2.72 ± 0.07 msec (S.D. 0.47 msec). Despite the introduction of a correction to a fixed distance no reduced dispersion of the observations made was thus obtained. When the correlation calculations were made a significant linear correlation between corrected delay and the concentration of plasma potassium was obtained, but not for any of the concentrations of non-protein nitrogen or the other serum electrolytes.

Corrected delay and plasma potassium

In 47 patients there was a positive connection between corrected delay and the plasma potassium concentration with the correlation coefficient $r = 0.35$ ($p < 0.02$) (fig. 21). The corresponding correlation coefficient for the uncorrected distal latencies was $r = 0.36$ ($p < 0.02$). An increase of 1 mEq per litre in the plasma potassium concentration caused a prolongation of 0.14 msec in the corrected delay.

Corrected delay — a composite function

Corrected delay is a composite function comprising both the conduction time between the stimulation point at the wrist corrected to 6.5 cm and the motor

Table 11. Motor nerve conduction velocity in 47 cases divided into groups according to the duration of the uraemia

Group	Number	Non-protein nitrogen mg per cent Mean value ± S.E.	Motor nerve conduction velocity m per sec Mean value ± S.E.	S.D.
No uraemia	7	35 ± 3	62.4 ± 2.8	7.5
Uraemia ≤ 1 month	22	191 ± 16	56.6 ± 1.7	8.2
Uraemia > 1 month	18	208 ± 14	52.4 ± 1.1	4.8

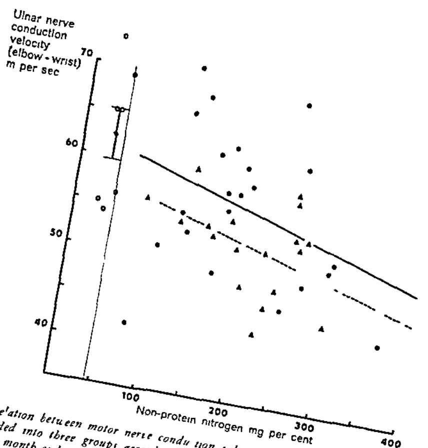


Fig 20 The relation between motor nerve conduction velocity and non protein nitrogen concentration divided into three groups according to the uraemia condition Cases with a uraemia duration of one month or less are marked with dots and a fully drawn regression line with the equation $y = -0\ 031\ x + 62\ 4$ Cases with a uraemia duration of more than one month are marked with triangles and a broken regression line with the equation $y = -0\ 029\ x + 58\ 4$ Cases without uraemia are marked with circles and the mean value $\pm$ S E $62\ 4 \pm 2\ 8$ m per sec

end plate and the delay in and distal to the end plate In Chapter IV E it was shown that the motor nerve conduction velocity was dependent on the non protein nitrogen concentration while it did not seem to be influenced by the electrolyte situation The above mentioned conduction time may therefore be assumed to be affected by the non protein nitrogen concentration but this did not show any significant correlation with corrected delay (correlation coefficient $r = 0\ 26$ $p > 0\ 10$) To obtain a simultaneous evaluation of the significance of the concentrations of non protein nitrogen and plasma potassium for corrected delay a multiple regression equation was calculated by means of the values for 42 patients in which both these factors were taken into account

Corrected delay $= 0\ 00063$ [N P N] + $0\ 101$ [K] + $2\ 132$

where corrected delay is expressed in msec and [N P N] is the non protein nitrogen concentration in mg per cent and [K] the plasma potassium concentration in mEq per litre The equation gave $R = 0\ 36$ and thus did not produce any greater accuracy in the prediction of corrected delay than the equation which only took account of the concentration of plasma potassium

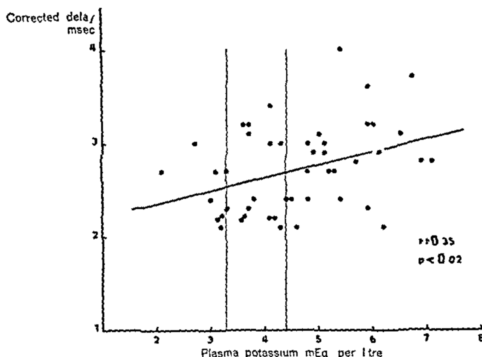


Fig 21 The relation between corrected delay and plasma potassium concentration The equation for the regression line is y = 0 14 x + 2 09

The multiple regression equation meant that an increase of 1 mEq per litre in plasma potassium with the non protein nitrogen concentration unchanged prolonged the corrected delay by 0 10 msec

G AMPLITUDE OF THE MUSCLE ACTION POTENTIAL UNDER NERVE STIMULATION

In 41 patients the amplitude of the action potential in the abductor digiti quinti muscle under ulnar nerve stimulation was on the average 17 6±1 0 mV (S D 6 4 mV) There were no significant linear correlations between these amplitudes and the concentrations of non protein nitrogen or serum electrolytes

H REPETITIVE NERVE STIMULATION

The average percentage deviations in the amplitudes of the last muscle action potentials compared with those for the first potentials under repetitive stimulation are shown in table 12 These did

Table 12 Average percentage change in amplitude in the muscle action potential under repetitive nerve stimulation at different frequencies in 40 patients

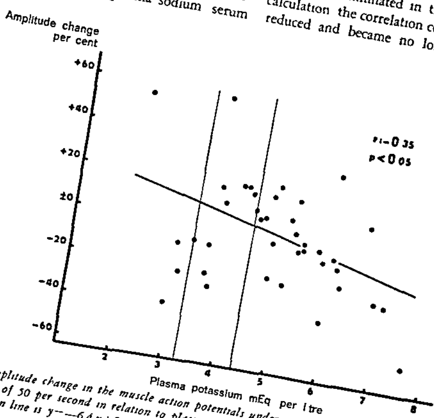
Number of stimuli per sec	Percentage change in amplitude Mean value±S E	S D
10	+2 7±2 2	13 9
30	+0 5±2 9	18 1
50	−3 0±3 7	23 1

not significantly deviate from zero at any of the stimulation frequencies The difference between the simultaneous recordings with two needle electrodes should with perfect technique be nil Despite the precautions taken there were great differences between the percentage changes of amplitude with two simultaneous recordings These differences were on the average 18 for the frequency of 10 per sec (59 examinations) 27 for the frequency of 30 per sec (56 examinations) and 29 for the frequency of 50 per sec (58 examinations) The mean value of the simultaneous recordings was used in the calculations made

There was no significant linear correlation between the percentage change in amplitude and the concentration of non protein nitrogen plasma sodium serum chloride standard bicarbonate base excess calcium inorganic phosphorus or magnesium

Repetitive nerve stimulation and plasma potassium

At the frequency of 50 per sec there was a correlation between the percentage change in amplitude of the muscle action potential and the concentration of plasma potassium (fig 22) whereby an increase of 1 mEq per litre in the plasma potassium concentration was connected with an average decrease in amplitude of 6 4 per cent ($p<0\ 05$) When one of the observations that is either that with the lowest or that with the highest potassium value was eliminated in the correlation calculation the correlation coefficient was reduced and became no longer signifi



? *Amplitude change in the muscle action potentials under repetitive nerve stimulation at* *…ency of 50 per second in relation to plasma potassium concentration The equation for* *…ression line is* $y = -6\ 4\,x + 28\ 6$

cant At lower stimulation frequencies there was no corresponding connection as the correlation coefficient for the relation between the percentage change of amplitude and the plasma potassium concentration at the frequency of 30 per sec was r = —0 11 (p>0 40) and at the frequency of 10 per sec r = —0 21 (p>0 10)

Post tetanic nerve stimulation

The percentage differences between the amplitudes of the muscle action potentials under post tetanic nerve stimulation and the amplitudes of the first muscle action potentials in the respective repetitive stimulation did not on the average significantly deviate from zero at any of the frequencies (table 13)

In the correlation testing between the percentage change of amplitude in the post tetanic potential and the concentration of non protein nitrogen or serum electrolytes there were no significant linear correlations

I MUSCLE BIOPSY

A biopsy was taken from the anterior tibial muscle on 32 occasions in 25 patients In the seven cases where two biopsies were taken one was taken from each anterior tibial muscle After histological examination all samples were considered to be normal musculature with two exceptions In one of these cases (EMG 567) atrophic muscle fibres were found arranged in groups at several points estimated by the muscle pathologist as indicating a neurogenous atrophy, and in the other case (EMG 552) certain muscle fibres showed signs of degeneration but there were no infiltrations of inflammatory cells Among 11 of the remaining 23 patients there were some isolated lymfocyte infiltrations (3 cases) isolated atrophic fibres (5 cases) and some fibres with centrally situated nuclei (4 cases) However in none of these 11 patients were the observed changes such that the musculature could be classified as pathological The electromyographic findings in the brachial biceps muscle in the two patients with the pathological findings and the 11 patients with the borderline findings in the muscle biopsies did not significantly differ from those in the remaining 12 patients

Muscle water content

Among 25 patients the total quantity of water (H_2O_m) in biopsies from the anterior tibial muscle was on the average 395 7±6 5 (S D 32 3) grammes per 100 grammes of fat free solids

Table 13 Average percentage change in amplitude in the muscle action potential with isolated nerve stimulation 1 12±0 01 sec after a repetitive stimulation compared with that for the first muscle action potential during the previous repetitive stimulation at different frequencies

Number of stimuli per sec in foregoing train	Percentage change in amplitude		
	Number	Mean value ± S E	S D
10	38	—1 7±1 1	6 5
30	39	—1 9±2 5	15 7
50	39	+2 3±1 9	11 6

H_2O_m was correlated with the change in amplitude under repetitive stimulation at a frequency of 30 per sec ($r = -0.45$ $p < 0.05$) and with the change in amplitude under isolated post tetanic stimulation after stimulation at a frequency of 30 per sec ($r = -0.57$, $p < 0.005$) If one single extreme value (EMG 552) was eliminated from the calculations however, the significance disappeared and the correlation coefficients became $r = -0.22$ ($p > 0.30$) and $r = -0.28$ ($p > 0.10$) respectively There were no significant correlations between H_2O_m and the electromyographic parameters

J EXAMINATIONS BEFORE AND AFTER TREATMENTS WITH THE ARTIFICIAL KIDNEY

Examinations were carried out on 17 patients immediately before and after a treatment with the artificial kidney on 19 occasions (table 14)

In connection with 4 treatments (3 patients) the patient was able to show an interference pattern with an amplitude of more than 1 5 mV at *maximal effort* before but not after the treatments With regard to the reduction in body weight non protein nitrogen and plasma potassium concentrations these 4 treatments did not differ from the others where in 10 cases the patient displayed an interference pattern with an amplitude of 1 5 mV or more both before and after the treatment and in 5 cases did not reach this contraction pattern

Spontaneous activity was not observed on any occasion in the examinations before the treatments but was recorded in one case after a treatment in the form of pseudomyotonic bursts

The *mean duration of motor unit potentials* tended to decrease during the treatments with the artificial kidney (fig 23) and the mean value for the differences before and after the treatments was -6.1 ± 2.2 per cent ($p < 0.02$)

The incidence of *polyphasic motor unit potentials* increased from 1.5 ± 0.3 per

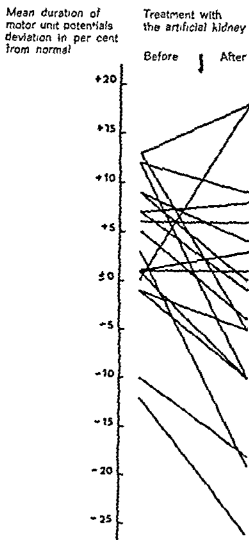


Fig 23 Mean duration of motor unit potentials before and after 19 treatments (17 patients) with the artificial kidney Mean value of the differences before and after the treatments was -6.1 ± 2.2 per cent deviation from normal ($p < 0.02$)

Table 14 Results with electromyography and motor nerve stimulation before and after treatments with the artificial kidney

	Number of treatments	Before treatment Mean value ± S E	After treatment Mean value ± S E	Mean difference ± S E	P
Mean duration of motor unit potentials per cent dev from normal	19	+3 9 ± 1 6	—2 2 ± 2 7	—6 1 ± 2 2	< 0 02
Polyphasic motor unit potentials per cent	19	1 5 ± 0 3	3 3 ± 0 6	+1 9 ± 0 7	< 0 02
Motor nerve conduction velocity m per sec	17	54 4 ± 1 1	55 0 ± 1 9	+0 6 ± 1 6	—
Corrected delay msec	12	2 93 ± 0 15	2 98 ± 0 21	+0 05 ± 0 16	—
Amplitude of muscle action potential mV	11	19 3 ± 1 6	15 1 ± 1 6	—4 1 ± 2 1	—

cent before the treatments to 3 3±0 6 per cent after the treatments The average increase was 1 8±0 7 per cent ($p<0.02$)

The *motor nerve conduction velocity* did not change during the treatments with the artificial kidney and was on average 54 4±1 1 m per sec before and 55 0±1 9 m per sec after the treatments

The *corrected delay* also remained unchanged and was 2 93±0 15 msec before and 2 98±0 21 msec after the treatments

The *amplitude* of the muscle action potentials at nerve stimulation did not change significantly during the treatments with the artificial kidney

There were no differences in the percentage changes in the amplitudes of the muscle action potentials under *repetitive nerve stimulation* and *post tetanic nerve stimulation* at any of the stimulation frequencies between the examinations made before and after the treatments with the artificial kidney

The *muscle water content* was examined in connection with 5 treatments and decreased during those treatments from 417±6 to 377±6 grammes per 100 grammes of fat free solids ($p<0.005$)

V DISCUSSION

The following discussion will mainly concern the principal findings of the investigation which in the case of the electromyographic examinations were that the mean duration of motor unit potentials was prolonged in patients with hyponatremia (Chap IV A) and that the incidence of spontaneous fibrillation potentials was highest in patients with the lowest plasma potassium and highest plasma sodium concentrations (Chap IV C) Under motor nerve stimulation it emerged that the motor nerve conduction velocity of the ulnar nerve in the forearm was lowest in patients with the highest uraemia, particularly if this was chronic (Chap IV E) and that the distal latency of the action potential in the abductor digiti quinti muscle under ulnar nerve stimulation at the wrist was prolonged in patients with hyperpotassemia (Chap IV F) Finally, the results of the examinations made before and after treatment with the artificial kidney (Chap IV J) will be discussed

The mean duration of motor unit potentials (Chap IV A) did not deviate significantly on the average from the normal values used The mean durations of motor unit potentials did not display any significant linear correlation with any of the serum electrolytes but were prolonged when the plasma sodium concentration was low A prolonged duration of the potentials may be due to the fact that an increased number of muscle fibres participate in the formation of the potential or that there is a temporal dispersion between the different muscle fibres in the motor unit The former situation can arise for example with a synchronised activity between different motor units while the number of fibres in the motor unit can scarcely be conceived in this connection to have been increased by sprouting of the nerve fibres as is seen in the case of partial nerve lesion causing an increase in the size of surviving motor units (Wohlfart, 1955) as the mean durations in the course of several days could both increase and decrease An increased temporal dispersion between the various muscle fibres may arise through a reduced conduction velocity in the distal nerve branches an increased delay in the end plates or prolonged propagation in the muscle fibres (Buchthal, 1962) Nastuk and Hodgkin (1950) recorded the intracellular action potential of frog muscle fibres under direct stimulation When the sodium concentration in the surrounding medium was reduced the amplitude of this potential fell Even if no quantitative results were obtained in this respect the recordings show that the rate of rise in the potential was reduced and that its duration was prolonged when the concentration of sodium was

reduced to 20—30 per cent of the normal concentration and replaced with choline The extracellular action potential, which closely resembled the second derivative of the intracellular action potential (Håkansson, 1957), probably also had a prolonged duration To some extent at least the prolonged mean duration of motor unit potentials in the case of hyponatremia could therefore be explained by a changed form of the action potentials of the individual muscle fibres Further examination with a multielectrode according to Buchthal et al (1957 a) could provide information on a possible altered expansion of the motor unit territory (Buchthal et al 1957 b) and with a multielectrode according to Ekstedt (1964) on a possible changed propagation velocity in the muscle fibres (Stålberg 1966)

Hausmanowa Petrusewicz et al (1962) found that the mean duration of motor unit potentials in 22 patients with acute renal failure and hyperpotassemia was reduced by 44 per cent (S D 9 per cent) in comparison with normal data in the same laboratory used in this study However there was no connection between the degree of hyperpotassemia and the extent of the reduction in the mean duration of motor unit potentials nor had the mean duration of motor unit potentials changed significantly but was shortened by 38 per cent (S D 11 per cent) when 14 patients were examined later 24—48 hours after they had obtained a normal plasma potassium concentration Only 1—3 months later when 5 patients were re examined had the mean duration of the motor unit potentials increased ($p<0\ 001$) and was shortened by 12 per cent (S D 10 per cent) The finding that there was no connection between the degree of hyperpotassemia and the degree of deviation from the normal value for the mean duration of motor unit potentials agrees with the finding in the present series The generally shortened mean duration of motor unit potentials could not however be confirmed However there was a certain difference between the two series that of Hausmanowa Petrusewicz et al (1962) merely comprising patients with acute renal failure Any difference in the degree of hyperpotassemia was not evaluated as Hausmanowa Petrusewicz et al (1962) only gave the plasma potassium concentrations for some of their patients and not for the whole group It is possible that the process that gave a prolonged distal latency with hyperpotassemia (Chap IV F) could have caused a partial blocking in the motor unit with a further increase in the potassium concentration A loss of active muscle fibres caused in this way would produce a shortened mean duration of the motor unit potentials (Kugelberg 1949) A more pronounced hyperpotassemia in the cases investigated by Hausmanowa Petrusewicz et al (1962) than in the present investigation could thus explain the differences in the mean duration of the motor unit potentials between the two studies It may be mentioned however that in the present series and in the group reported by the authors mentioned above there were several cases with changes in the electrocardiogramme of the hyperpotassemic type

In the present series the *incidence of polyphasic motor unit potentials* was $2\ 7 \pm 0\ 4$ per cent (Chap IV B) Buchthal et al (1954 a) found an incidence of polyphasic motor unit potentials of 3 4 per cent in the brachial biceps muscle in normal subjects from 20 to 22 years of

age Sacco et al (1962) recorded an incidence of polyphasic potentials of 2 8 per cent for the same muscle in subjects from 16 to 23 years of age and 3 8—4 9 per cent in higher age groups With decreasing intramuscular temperature the incidence of polyphasic potentials increased and in the temperature range down to 30° C was 10—15 per cent (Buchthal et al 1954 b) In the present study the intramuscular temperature in the brachial biceps muscle varied within a small range (23 patients 36 2±0 1°C S D 0 6° C) and there was no correlation with the incidence of polyphasic motor unit potentials Hausmanowa Petrusewicz et al (1962) in their study of patients with acute renal failure found a great number of polyphasic units in 5 cases out of 22 with hyperpotassemia but this was not described in greater detail The incidence of polyphasic motor unit potentials in the present study could not be correlated with the concentration of plasma potassium but on the other hand it tended to increase with rising serum calcium concentration The degree of significance depended however on too few cases of hypercalcemia and therefore the observation although a point to be noted did not permit any conclusions

Hypocalcemia has been described as causing *spontaneous repetitive activity* (Adrian and Gelfan 1933) In the present study the groups with spontaneous activity did not differ in respect of the serum calcium concentration from the group without such activity (Chap IV C) However it must be pointed out that it is not the total calcium concentration which has been determined here but also the degree of ionization of calcium which is probably significant No attempt was made to calculate the degree of ionization of calcium as Fanconi and Rose (1958) have in fact shown that the quantity of ionized calcium cannot be predicted in uraemic cases even if the total calcium concentration the plasma protein concentration and the blood pH are known and that the only sure method is to make direct measurements which was not done in the present study

Buchthal and Rosenfalck (1966) found spontaneous fibrillation potentials at one point in 7 of 197 brachial biceps muscles (3 6 per cent) in subjects without signs or symptoms of neuromuscular disorders In the present study spontaneous fibrillation potentials were recorded at one point in the muscle in 8 cases and at two points in the muscle in 2 cases out of 109 examinations (a total of 9 2 per cent) There was no significant difference between these incidences ($p>0\ 05$)

Solandt and Magladery (1940) found that in the denervated gastrocnemius muscle in rats the administration of potassium chloride temporarily stopped the fibrillations Hall and Knox (1952) found in investigating spontaneous fibrillation in isolated rat diaphragm preparation after denervation that the fibrillations became greatly enhanced if the potassium content in the surrounding solution was reduced from 5 88 to 1 17 mEq per litre

Recording miniature end plate potentials in frog muscle Birks (1963) showed that the introduction of digoxin into the surrounding substrate (Ringer solution) caused an increase of amplitude and frequency During a control period the frequency was usually less than 1 per sec after 50—70 min it was 40—100 per sec At this frequency spontaneous muscle action potentials also occurred Elmqvist and Feldman (1965) made similar obser

vations after adding ouabain to rat diaphragm preparation Birks (1963) considered that this increased outflow of acetylcholine observed was caused by an increased intraneural sodium concentration produced by the blocking effect of digoxin on the sodium pump In the group with spontaneous fibrillation potentials in the present study there was no treatment with digitalis in any case but a low plasma potassium concentration which produced a tendency to increased intracellular sodium concentration (Cooke and Segar, 1952), thus an effect similar to that obtained by blocking the sodium pump The higher plasma sodium concentration in this group may be assumed to accentuate this effect If this was the cause of the fibrillation potentials they would thus be released via the nerve terminals and not directly in the muscle membrane Riecker et al (1964) working with patients with hypopotassemia due to a lack of potassium found when individual muscle cells were punctured that the resting potential of the muscle membrane was higher than normal which would rather cause a reduced excitability in the membrane (Jenerick and Gerard 1953)

In the group with spontaneous fibrillation potentials the concentration of plasma potassium was on average 3 5±0 3 mEq per litre and of plasma sodium 139±3 mEq per litre These mean values fall within the normal range for the respective electrolyte concentrations The spontaneous fibrillation potentials recorded in normal subjects may therefore be thought possibly to arise owing to small electrolyte disturbances within the normal range

In one patient there was a profuse spontaneous activity which may have been induced by a hypomagnesemia (De Doncker and Rosselle, 1959) as well as hypopotassemia

Rosselle and De Doncker (1961) found both fibrillar activity and myotonic volleys in 2 patients with hypopotassemia and alkalosis, the latter of which seemed to them typical of hypopotassemia In the present study pseudomyotonic bursts were recorded in 5 patients The serum electrolytes for these patients did not however differ significantly from those for the remaining patients without such activity

In an electromyographic examination of patients with acute renal failure Hausmanowa Petrusewicz et al (1962) found when recording the maximal efforts of 22 patients with hyperpotassemia that about half of them displayed a sparse electromyographic pattern or reduced amplitude In the present study 18 patients with a mixed pattern or a pattern of single motor unit potentials or an amplitude below 1 5 mV at *maximal voluntary* contraction had on the average a higher plasma potassium value (6 0±0 4 mEq per litre) than the 47 patients who were able to display an interference pattern and an amplitude of 1 5 mV or more, for which the average plasma potassium concentration was significantly lower (4 4±0 2 mEq per litre) (Chap IV D) There were however other significant differences between the two groups of patients as the hyperpotassemic group in addition to a higher non protein nitrogen concentration also had a more pronounced metabolic acidosis and hyperphosphatemia than the other group Thus the inferior activity pattern could not be attributed to hyperpotassemia but could just as well have been due to a more extreme uraemic intoxication or metabolic acidosis When recording

maximal voluntary contraction good co operation is required from the patient which was not always the case with highly uraemic patients This lack of co operation was certainly not always due to uraemia and electrolyte disturbances but also to other circumstances such as a poor general condition caused by infections vomiting etc Thus many different factors could have affected the activity pattern and probably no safe conclusions can be drawn from these investigations in this matter

The motor conduction velocity in the ulnar nerve in the forearm was on the average in this study 55 9 ± 1 0 m per sec (Chap IV E) which does not differ very much from the value reported after investigations of normal individuals (i a Henriksen 1956 Thomas et al 1959 Mayer 1963 Trojaborg 1964) In analysing the connection between the motor nerve conduction velocity and the degree of uraemia and the serum electrolyte concentrations significant correlations were obtained indicating that the motor nerve conduction velocity was slowed down with increasing non protein nitrogen and plasma potassium concentrations It could however be proved that the most important of these two factors was non protein nitrogen while the plasma potassium concentration did not seem to affect the motor nerve conduction velocity This latter fact has also been observed by Simpson (1962) who found a normal ulnar nerve conduction velocity in 4 patients with hypopotassemia and 2 with hyperpotassemia An increase of 100 mg cent in the non protein nitrogen concentration caused a reduction of about 4 0 m per sec in the motor nerve conduction velocity Parts of the present material have been preliminarily reported earlier (Lindholm 1966) These findings are in agreement with those which Jebsen et al (1967) reported after repeated examinations on 14 uraemic patients where a rising serum creatinine concentration was connected with a fall in the motor nerve conduction velocity The results of the present investigation indicate that the motor nerve conduction velocity does not only decrease with increasing uraemia (expressed as non protein nitrogen concentration) but also with the duration of the uraemia

The distal latency under stimulation of the ulnar nerve at the wrist and recording of the action potential in the abductor digiti quinti muscle corrected for the distance was called corrected delay and was on the average in 47 cases 2 72 ± 0 07 msec (Chap IV F) This did not differ from what had been reported previously after investigations of normal persons (Carpendale 1956 Mavor and Libman 1962) The correlation tests showed that corrected delay increased with rising plasma potassium concentraton Corrected delay is a complex quantity covering both the propagation in the motor nerve and a delay in and distal to the end plate As was shown in the earlier discussion the motor nerve conduction velocity in the ulnar nerve in the forearm was affected by the concentration of non protein nitrogen but not that of plasma potassium Thus the dependence on potassium is probably to be found in or distally to the nerve terminals An increased interval between the nerve stimulation and the muscle response in the case of hyperpotassemia has previously been observed in experiments on rats (Walker 1948) and rabbits (Gamstorp and Vinnars 1961)

Both the nerve conduction velocity and the distal latency depend on temperature Henriksen (1965) showed that the conduction velocity in the ulnar nerve decreased approximately 2 4 m per sec when the temperature fell by 1° C within the range 29 to 38° C Abramson et al (1966) found that when the forearm was heated in a water bath the ulnar nerve conduction velocity increased by 3 5 m per sec when the temperature in the brachioradialis muscle rose from 35 3 to 36 9° C which corresponded to 2 2 m per sec per ° C Carpendale (1956) found that the distal conduction time on stimulation of the ulnar nerve and recording of the action potential in the abductor digiti quinti muscle increased 0 2 msec per ° C of reduction in temperature in the range 25 to 35° C Unfortunately no temperature measurements were made in the ulnar nerve in the present investigation nor were any measures taken to control the temperature in the extremities examined The temperature in the brachial biceps muscle was not significantly correlated with the concentration of non protein nitrogen or plasma potassium nor were the patients rectal temperatures correlated with these concentrations The temperature in the forearm is lower than that in the upper arm (Buchthal et al 1944 Trojaborg 1964) therefore the temperatures measured in the brachial biceps muscle cannot be regarded as representative for the temperature in the ulnar nerve in the forearm and hand A reduced peripheral circulation in the patients with high uraemia in the present investigation could be considered as giving these patients a reduced peripheral temperature compared with the other patients An arterial blood pressure lower than 100 mm Hg was found in one patient only (EMG 521) in whom the motor nerve conduction velocity was 54 m per sec and corrected delay 3 1 msec An examination of the patients circulation conditions showed that an insufficient or suspected insufficient circulation despite the fact that the blood pressure was not low occurred in a further 4 of the 54 patients for whom the ulnar nerve conduction velocity was determined When these 5 patients were eliminated from the calculations there was no significant change in the results obtained, neither in the correlation between the non protein nitrogen concentration and the motor nerve conduction velocity nor that between the plasma potassium concentration and corrected delay

The amplitude of the abductor digiti quinti muscle action potential under ulnar nerve stimulation was on the average 17 6±1 0 mV (Chap IV G), which was close to Trojaborg s findings (1964) of 16 8±0 8 mV for the corresponding amplitude Walker (1948) and Gamstorp and Vinnars (1961) found with animal experiments that the muscle action potential under nerve stimulation had a reduced amplitude and a prolonged duration in cases of hyperpotassemia In the present investigation no significant reduction in amplitude could be recorded in the case of hyperpotassemia The duration of the stimulation response was not measured as the terminal components of the action potential very often could not be determined with a sufficient degree of accuracy

With *repetitive stimulation* for 1 second there were no systematic changes in amplitude at any of the stimulation frequencies of 10 30 or 50 per sec (Chap IV H) At the stimulation frequency of

50 per sec a correlation was observed where the amplitude of the action potential in the abductor digiti quinti muscle during the repetitive nerve stimulation decreased with increasing plasma potassium concentration However the significance was low and disappeared if a few extreme values were eliminated from the calculations

Grob et al (1957) investigated the amplitude of the action potential in the adductor pollicis brevis muscle under single nerve stimulus after tetanic stimulation of the ulnar nerve (25 or 50 per sec for 10 sec) After ingestion of potassium chloride this amplitude was in 4 subjects unchanged or slightly reduced After tetanic stimulation of the motor nerve Brown and von Euler (1938) with cats and Walker (1948) with rats found a reduction in the amplitude of the muscle action potential under single stimulations and suggested that this was caused by the potassium mobilisation occuring during muscle contraction (Fenn and Cobb 1936 Grob et al 1957) No *post tetanic changes in amplitude* were recorded in the present investigation However during the repetitive stimulation the frequencies were lower or the duration shorter than in the latter authors experiments which meant that a more permanent extracellular potassium increase possibly was not produced

The plasma sodium and plasma potassium concentrations thus influenced the function in the motor unit indicating that both of these ion concentrations could be relevant to the presence of muscular weakness in patients with renal insufficiency The degree of uraemia expressed as non protein nitrogen concentration affected the motor nerve conduction velocity but did not seem to affect other parts of the motor unit

Several disturbances in the electrolyte fluid balance were corrected within a short time during the *treatments with the artificial kidney* (Chap IV J) A significant reduction was observed for the concentrations of non protein nitrogen plasma potassium inorganic serum phosphorus and serum magnesium while the standard bicarbonate concentration rose The muscle water content examined before and after 5 treatments, showed a significant reduction This dehydration of the muscle tissue was caused to a certain extent by the ultrafiltration effect achieved with the type of artificial kidney used and which gave a total dehydration and decreasing body weight in the patients To a certain extent the decreasing muscle water content could also have been caused by a re distribution of water to other organic systems, such as the central nervous system (Dossetor et al 1965) in which the urea concentration decreases more slowly than in serum in connection with treatment with the artificial kidney (Kennedy et al 1962) In a comparison of the electromyographic examinations made before and after 19 treatments with the artificial kidney it emerged that the mean duration of motor unit potentials decreased and that the incidence of polyphasic motor unit potentials increased, while none of the parameters registered during motor nerve stimulation was changed It was shown above that the concentration of plasma sodium was the only plasma or serum electrolyte concentration that affected the mean duration of the motor unit potentials Since the plasma sodium concentration did not change during the treatments the short

ened mean duration of motor unit potentials could probably not be explained on the basis of the concentrations of the serum electrolytes The ulnar motor nerve conduction velocity and the corrected delay were unchanged during the treatments, therefore the reduced potential duration could similarly probably not be explained by a smaller temporal dispersion between the separate muscle fibres in the motor units caused by a faster propagation in the distal nerve branches From 417 grammes per 100 grammes of fat free solids before the treatments the muscle water content fell by about 10 per cent If this dehydration was assumed to be of the same form in the extra and intracellular spaces, it would mean a reduction of about 5 per cent in the muscle fibre diameters Such a small geometric change probably caused no noticeable alteration in the propagation velocity of the action potential in the muscle fibre (Buchthal et al, 1955), but if it did the velocity should have been reduced (Håkansson, 1956) The electromyographic changes observed in connection with the treatments with the artificial kidney must therefore be left unexplained The combination of a decreasing mean duration of motor unit potentials with an increasing incidence of polyphasic motor unit potentials may indicate that a loss of muscle fibre had occurred It must be pointed out however that a dialysis treatment does not only correct disturbances in the electrolyte balance and reduce the serum concentration of non protein nitrogen but also interferes in the metabolic disturbances that occur in uraemia and which are not fully known (Leiter, 1950, Schreiner and Maher, 1961)

VI SUMMARY

In patients with renal insufficiency muscular weakness may occur not only from effects on the central nervous system but also from effects on the peripheral neuro muscular system In recent years in particular since regular dialysis treatment has become a generally accepted method of treatment for patients with a chronic irreversible renal insufficiency several reports have been published regarding neuropathy in such patients including measurement of nerve conduction velocities Findings of myopathy in patients with chronic renal insufficiency have also been reported Sometimes however, a muscular weakness in patients with renal insufficiency can be explained by disturbances in the electrolyte balance In reports on this subject the potassium ion has taken a central place Electromyographic examination of patients with renal insufficiency with simultaneous determination of serum electrolytes seems to have hitherto only been carried out by Hausmanowa Petrusewicz et al (1962) These authors found an average shortened mean duration of motor unit potentials in 22 cases with hyperpotassemia and in about half of the cases with maximal effort recording a sparse electromyographic pattern or reduced amplitude but owing to the fact that the experimental conditions were of a very complex nature they drew no final conclusions

The purpose of the present study was to attempt to describe how disturbances in the electrolyte fluid balance affect the neuromuscular function

Electromyography in the brachial biceps muscle (109 examinations 65 patients) and ulnar motor nerve stimulation (90 examinations 55 of the patients) with simultaneous determination of the uraemia and electrolyte conditions were carried out on patients with renal diseases Fifty five of the patients were uraemic and with few exceptions all had one or more abnormal serum electrolyte concentrations

The mean duration of motor unit potentials was prolonged in cases with hyponatremia but in other respects there were no significant correlations with concentrations of non protein nitrogen or serum electrolytes

Spontaneous activity in the form of fibrillation potentials occurred in 10 examinations in which compared with the remaining examinations there was a lower concentration of plasma potassium and a higher concentration of plasma sodium and serum chloride

The motor nerve conduction velocity in the ulnar nerve in the forearm decreased with increasing uraemia expressed as the non protein nitrogen concentration and was most reduced in a patient group

with a uraemia duration of more than a month

The distal latency on stimulation of the ulnar nerve at the wrist with recording of the action potential in the abductor digiti quinti muscle was *prolonged in* cases of hyperpotassemia

The polyphasic motor unit potentials displayed a tendency to increase in incidence with rising serum calcium concentration The significance of the correlation was however dependent on too few cases of hypercalcemia

The pattern at maximal voluntary contraction and the amplitudes of the abductor digiti quinti muscle action potential under single repetitive and post tetanic ulnar nerve stimulation did not permit any conclusions

During treatment with the artificial kidney the mean duration of motor unit potentials decreased and the incidence of polyphasic motor unit potentials increased The muscle water content was reduced and simultaneous changes in blood concentration were a decrease in *non protein nitrogen, plasma potassium* inorganic serum phosphorus and serum magnesium and an increase in standard bicarbonate None of these observed changes in the electrolyte fluid balance could however explain the electromyographic findings

ACKNOWLEDGEMENTS

The present study was carried out in the Medical Department B (Renal Clinic) University of Lund Lund Sweden during the years 1961—1967

I wish to express my sincere gratitude to Professor Nils Alwall for generous help advice and encouragement throughout the investigation

Professor Fritz Buchthal for his valuable teaching constructive criticism and experienced advice

Mr Poul Rosenfalck M Sc for generous and never tiring help as well as invaluable discussions

Professor Carl Gottfrid Holmberg for kindly allowing me to publish data from his laboratory

Dr Karl Gosta Henriksson for the preparation and description of the muscle biopsies

Fil kand Arne Frennelius for the selection and formulation of the statistical methods

Docent Ingrid Gamstorp Docent Håkan Håkansson and Docent David Ingvar for stimulating discussions

Mr Joseph Szalai for skilful technical assistance

This investigation was supported by grants from the Swedish Medical Research Council (to Professor Nils Alwall project B68 19X 763 03) from the Medical Faculty of the University of Lund Sweden from the Swedish Life Insurance Companies Committee for Medical Research from the Marie Louise Schele s Memorial Fund and from Ebba and Herman Bing s legacy

REFERENCES

Abram on D I Chu L S W T k S Jr Lee S W R chard G & Le n M Effect of t ssue temperatures and blood flow on motor nerve conduct on veloc ty J Amer med Ass *198* 108 1966

Adr an E D & Gelfan S Rhythm act t n skeletal muscle f bres J Phys ol (Lond) *78* 271 1933

Alu all N Therapeut c and d agnost c problems in severe renal fa lure Scand nav an Un vers ty Books Stockholm 1963

Andersen O S & Engel K A new ac d base nomogram An impro ed method for the calculat on of the relevant blood ac d base data Scand J cl n Lab Invest *12* 177 1960

A bury A K V ctor M & Adams R D Urem c polyneuropathy Arch Neurol (Ch c) *8* 413 1963

Bergstrom J Muscle electrolytes n man Scand J cl n Lab Invest *14* suppl 68 1962

B rks R I The role of sod um ons n the metabol sm of acetylchol ne Canad J B ochem *41* 2573 1963

Botelho S Y & Cander L Post tetan c po tent at on before and dur ng ischem a n ntact human skeletal muscle J appl Phys ol *6* 221 1953

Broun G L & vo Euler U S The after effects of a tetanus on mammal an muscle J Physiol (Lond) *93* 39 1938

B own M R Currens J H & Marchand J F Muscular paralys s and electrocar d ograph c abnormal ties result ng from potass um loss in chronic nephr t s J Amer med Ass *124* 545 1944

Buchthal F An ntroduct on to electromyo graphy Scand nav an Un ers ty Books Copenhagen 1957

B chthal F The electromyogram Wld Neurol *3* 16 1962

Bu htlal F Guld C & Rosenfalck P Act on potent al parameters in normal human muscle and the r dependence on phys cal var ables Acta phys ol scand *3* 00 1954 a

Bu h hal F Guld C & Ro enfalck P In ne at on zone and propagat on veloc ty n human muscle Acta phys ol scand *35* 1 4 1955

B hthal F Guld C & Rosenfalck P Vo lume onduct n of the sp ke of the motor un t potent al n estigated with a new type of mult electrode Acta phys ol scand *38* 331 1957 a

B h hal F Guld C & Ro enfalck P Mul t ele trode tudy of the territory of a m tor un t Acta phys ol scand *39* 83 1957 b

B hthal F Honcke P & Lundard J Tem perature measurements in human muscles n tu at rest and dur ng muscular work Acta phys ol scand *8* 230 1944

Bu h hal F P nell P & Rosenfalck P Act on potent al parameters n normal human muscle and the r phys ological determ nants Acta phys ol scand *32* 219 1954 b

Bu hthal F & Rosenfalck P Spontaneous electrical activ ty of human muscle Elec troenceph cl n Neurophys ol *20* 321 1966

B ll G M Carter A B & Lowe K G Hyperpotassaem c paralys s Lancet *2* 60 1953

Callaghan N Restless legs syndrome in ure m c neuropathy Neurology (M nneap) *16* 359 1966

Carpendale M T F Conduct on time in the term nal port ons of the motor nerve f bres

of the ulnar median and peroneal nerves in healthy subjects and patients with neuropathy Thesis University of Minnesota 1956

Caruso G & Buchthal F Refractory period of muscle and electromyographic findings in relatives of patients with muscular dystrophy Brain *88 29 1965*

Charcot J M Des paraplégies urinaires Leçons sur les maladies du systeme nerveux A Delahaye et Lecrosnier Paris 1880

Conway E J & Fearon, P J The acid labile CO in mammalian muscle and the pH of the muscle fibre J Physiol (Lond) *103* 274 1944

Cooke R E & Segar W E A proposed mechanism of extracellular regulation of muscle composition Yale J Biol Med *25* 83 1952

Croft P B & Wilkinson M The incidence of carcinomatous neuromyopathy in patients with various types of carcinoma Brain *88* 427 1965

Dinapoli R P Johnson W J & Lambert E H Experience with a combined hemodialysis renal transplantation program neurological aspects Mayo Clinic Proc *41* 809 1966

De Doncker K & Rosselle N Étude de l activite myoelectrique dans la déficience magnesique experimentale J Physiol (Paris) *51* 39 1959

Dossetor J B Oh J H Dayes L & Pappius H M Brain urea and water changes with rapid hemodialysis of uremic dogs Trans Amer Soc Artif Intern Organs *10* 323 1964

Ekstedt J Human single muscle fiber action potentials Acta physiol scand *61* suppl *226* 1964

Elmqvist D & Feldman D S Effects of sodium pump inhibitors on spontaneous acetylcholine release at the neuromuscular junction J Physiol (Lond) *181* 498 1965

Fanconi A & Rose G A The ionized complexed and proteinbound fractions of calcium in plasma An investigation of patients with various diseases which affect calcium metabolism with an additional study of the role of calcium ions in the prevention of tetany Quart J Med *27* 463 1958

Fenn W O & Cobb D M Electrolyte changes in muscle during activity Amer J Physiol *115* 345 1936

Finch C A & Marchand, J F Cardiac arrest by the action of potassium Amer J med Sci *206* 507 1943

Finch C A Sawyer C G & Flynn J M Clinical syndrome of potassium intoxication Amer J Med *1* 337 1946

Forno L & Alston W Uremic polyneuropathy Acta neurol scand *43* 640 1967

Funck Brentano J L Chaumont P Mery J Ph Vantelon J & Zinkgraff Kok J Interet de la mesure de la vitesse de conduction nerveuse dans la surveillance des malades uremiques soumis a des hemodialyses repetees Proc European Dialysis and Transplant Ass 2 23 1964

Gamstorp I & Vinnars E Studies in neuromuscular transmission II Influence on transmission of hyperpotassemia Acta physiol scand *53* 151 1961

Gomori G A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter J Lab clin Med *27* 955 1942

Grob D Liljestrand A & Johns R J Potassium movements in normal subjects Effect on muscle function Amer J Med *23* 340 1957

Hall J E & Knox J A C Fibrillation in the isolated rat diaphragm preparation after denervation J Physiol (Lond) *116* 29P 1952

Harvey A M & Masland R L A method for the study of neuromuscular transmission in human subjects Bull Johns Hopk Hosp *68* 81 1941

Hausmanowa Petrusewicz I Emeryk B & Lao M Bioelectric activity of skeletal muscles in renal failure with hyperpotassemia Pol Arch Med wewnet *32* 110 1962

Henriksen J D Conduction velocity of motor nerves in normal subjects and in patients with neuromuscular disorders Thesis University of Minnesota 1956

Herman E & Rado J Fatal hyperkalemic paralysis associated with spironalactone Arch Neurol (Chic) *15* 74 1966

Håkansson C H Conduction velocity and amplitude of the action potential as related to circumference in the isolated fibre of frog muscle Acta physiol scand 37 14 1956

Håkansson C H Action potentials recorded intra and extracellularly from the isolated frog muscle fibre in Ringer s solution and in air Acta physiol scand 39 291 1957

Jebsen R H Tenckhoff H & Honet J C Natural history of uremic polyneuropathy and effects of dialysis New Engl J Med 277 327 1967

Jenerick H P & Gerard R W Membrane potential and threshold of single muscle fibers J cell comp Physiol 42 79 1953

Kemp T & Nielsen A Statistik for medicinere Munksgaard Copenhagen 1961

Kennedy A C Linton A L & Eaton J C Urea levels in cerebrospinal fluid after haemodialysis Lancet 1 410 1962

Kolff W J Serum potassium in uremia Report of sixteen cases some with paralysis J Lab clin Med 36 719 1950

Konotey Ahulu F I D Baillod R Comty C M Heron J R Shaldon S & Thomas P K Effect of periodic dialysis on the peripheral neuropathy of end stage renal failure Brit med J 2 1212 1965

Kugelberg E Electromyography in muscular dystrophies differentiation between dystrophies and chronic lower motor neurone lesions J Neurol Neurosurg Psychiat 12 129 1949

Kuchel O Kandrac M Kapitola J & Chartat J Einige Probleme der Pathogenese hypokalamischer Muskellahmungen Endokrinologie 41 129 1961

Leiter L Uremia Cleveland Clin Quart 17 49 1950

Lindholm T Electromyographic examinations of uraemic patients Proc European Dialysis and Transplant Ass 3 35 1966

Mavor H & Libman I Motor nerve conduction velocity measurement as a diagnostic tool Neurology (Minneap) 12 733 1962

Maudsley C & Mayer R F Nerve conduction in alcoholic polyneuropathy Brain 88 335 1965

Mayer R F Nerve conduction studies in man Neurology (Minneap) 13 1021 1963

Merrill J P The treatment of renal failure Therapeutic principles in the management of acute and chronic uremia W Heinemann Medical Books London 1965

Merrill J P, Levine H D Somerville W & Smith S Clinical recognition and treatment of acute potassium intoxication Ann intern Med 33 797 1950

Mollaret P Goulon M & Tournilhac M Contribution a l etude des paralysies avec hyperkaliemie II Les paralysies avec hyperkaliemie au cours de l insuffisance renale Rev neurol 99 241 1958

Mulder D W Lambert E H Bastron J A & Sprague R G The neuropathies associated with diabetes mellitus Neurology (Minneap) 11 275 1961

Nastuk W L & Hodgkin A L The electrical activity of single muscle fibers J cell comp Physiol 35 39 1950

McNaughton R A & Birchell H B Paralysis with potassium intoxication in renal insufficiency J Amer med Ass 145 481 1951

Nielsen V K Sensory nerve conduction studies in uraemic patients Proc European Dialysis and Transplant Ass 4 279 1967

Poinso R Toga M Murisasco A Regis H & Jauffret P Le muscle uremique Lyon med 213 479 1965

Preswick G & Jeremy D Subclinical polyneuropathy in renal insufficiency Lancet 2 731 1964

Radner S Instruments for percutaneous muscle biopsy Journal Stille Werner 21 Stockholm 1965

Ramelli E & Zerbi F Vitesse de conduction du nerf cubital dans l alcoolisme chronique (sur l incidence des alterations pre n vritiques) Electroenceph clin Neurophysiol suppl 22 43 1962

Rao C R Advanced statistical methods in biometric research J Wiley & Sons New York 1962

Rappaport F Rapid microchemical methods for blood and C.S F examinations Grune & Stratton, New York 1949

Richardson G O & Sibley J C Flaccid quadriplegia associated with hyperpotassemia Canad med Ass J 69 504 1953

Riecker G Bolte H D & Rohl D Hypokaliämie und Membranpotential Mikro-

punktionen einzelner Muskelzellen beim Menschen Reanimation et organes artificiels *1* 41 1964

Rosselle N & De Doncker K Étude electromyographique de deux cas d hypopotassemie et alcalose Acta clin belg *16* 332 1961

Sacco G Buchthal F & Rosenfalck P Motor unit potentials at different ages Arch Neurol (Chic) 6 366 1962

Scharter D Fluorometric estimation of mag nesium with 8 hydroxi 5 quinolin sulfonate J Lab clin Med *58* 495 1961

Schreiner G E & Maher J F Uremia biochemistry pathogenesis and treatment Charles C Thomas Publisher Springfield Ill 1961

Scribner B H Bedside determination of chloride a method for plasma urine and other fluids and its application to fluid balance problems Mayo Clinic Proc *25* 209 1950

Scribner B H Caner J E Z Burt R & Quinton W The technique of continuous hemodialysis Trans Amer Soc Artif Intern Organs 6 88 1960

Simpson J A Conduction velocity of peripheral nerves in human metabolic disorders Electroenceph clin Neurophysiol *suppl* 22 36 1962

Skillman T G Johnson F W Hamwi G J & Driskill H J Motor nerve conduction velocity in diabetes mellitus Diabetes *10* 46 1961

Smillie W G Potassium poisoning in nephritis Arch intern Med *16* 330 1915

Snedecor G W Statistical methods Applied to experiments in agriculture and biology AMES Iowa USA 1956

Solandt D Y & Magladery J W The relation of atrophy to fibrillation in denervated muscle *Brain* 63 255 1940

Stalberg E Propagation velocity in human muscle fibers in situ Acta physiol scand *70* suppl 287 1966

Tenckhoff H A Boen F S T Jebsen R H & Spiegler J H Polyneuropathy in chronic renal insufficiency J Amer med Ass *192* 1121 1965

Thomas P K Sears T A & Gilliat R W The range of conduction velocity in nomal motor nerve fibres to the small muscles of the hand and foot J Neurol Neurosurg Psychiat *22* 175 1959

Trojaborg W Motor nerve conduction velocities in normal subjects with particular reference to the conduction in proximal and distal segments of median and ulnar nerve Electroenceph clin Neurophysiol *17* 314 1964

Versaci A A Olsen K J McMain P B Nakamoto S & Kolff W J Uremic polyneuropathy and motor nerve conduction velocities Trans Amer Soc Artif Intern Organs *10* 328 1964

Walker S M Action potentials in rat muscle with twitch tension potentiated by KCl treatment adrenalectomy tetanus and treppe Amer J Physiol *154* 63 1948

Wohlfart G Aktuelle Probleme der Muskelpathologie Dtsch Z Nervenheilk *173* 426 1955

APPENDIX
(Tables)

Examination		38	39	176	D 119	D 120	D 122	D 136	D 137	D 138	D 112	D 143	D 144
Age		24	24	25	20	20	20	46	46	46	34	34	34
Sex		F	F	F	F	F	F	M	M	M	F	F	F
Duration of uraemia > 1 month		no	0	0	0	no	0	no	no	no	no	no	no
Non protein nitrogen mg%		51	21		45	66	28	157	153	111	189	104	154
Sodium mEq/l		168	141		157	112	138	135	110	138	129		128
Potassium mEq/l		1 6	2 9	3 2	3 7	3 0	2 6	5 2	6 5	3 8	5 0		5 8
Chloride mEq/l		100	111		107	94	94	66	95	96	99	92	86
Standard bicarbonate mEq/l		22 3	19 9		23 8	27 9	29 2	15 1	15 4	17 9	15 1	18 2	18 7
Base excess mEq/l		—1 0	—4 0			+6 0	+8 0						—6 0
Calcium mEq/l					5 9	1 7	5 3	4 0					
Inorg phosphorus mg%					1 8	3 8	3 1	8 8	11 2	6 5	6 1	4 9	
Magnesium mEq/l					2 0	1 7	1 7						
Serum protein g%		8 4	6 9		5 6		6 9	6 9	6 6	6 7	6 2	6 6	6 3
Interference pattern and amplitude ≥ 1 5 mV		+	+	+	—	—	+	+	+	—	+	—	—
Spontaneous fibrillation potentials		0	0	0	0	0	0	0	0	0	0	0	0
Motor unit potentials	Number	30	45	51	47	55	12	76	62	61	52	83	72
	Mean duration msec	12 6	11 5	11 3	11 2	10 9	12 8	10 5	11 5	9 1	10 7	10 3	10 7
	% dev from normal	+24	+13	+10	+12	+9	+28	—6	+3	—19	—1	—5	—1
	% polyphasic pot	0	4	8	0	5	5	5	3	2	4	7	1
Motor nerve conduction velocity m/sec				68	56	64	59	6[illegible]	58	57	61		45
Distal latency msec				2 3	2 2	2 6	2 6		3 0		3 0		2 8
Corrected delay msec				2 2	2 3	2 7	2 7		3 1		3 1		3 1
Total amplitude mV				26	17 5	16 5	21		22		30		27

Examination		38	39	126	119	D	120	122	136	137	D	138	112	D	113	114
Repetitive stimulation % amplitude change	10/sec															
	30/sec															
	50/sec															
Post tetanic stimulation % amplitude change	10/sec															
	30/sec															
	50/sec															
Intramuscular temperature °C musc biceps brachii																
Muscle water g/100 g FFS musc tibialis anterior																

* Examinations carried out on the same *patient* are in this and the *following* tables grouped by a line *drawn* under the numbers of the examinations D between *two* examinations indicates that the examinations were carried out before and after a treatment with the artificial kidney

Examination	147	148	152	155	156	177	180	185	188	163	176	179
	D					D		D			D	
Age	30	30	30	30	19	19	19	19	19	48	48	48
Sex	M	M	M	M	M	M	M	M	M	M	M	M
Duration of uraemia > 1 month	no	no	no	no	yes	yes	yes	yes	yes	yes	yes	yes
Non protein nitrogen mg%	173	115	55	24	256	213	117	194	100	240	276	129
Sodium mEq/l	[illegible]	117	132		138	120	129	132	130	133	119	124
Potassium mEq/l	4 [illegible]	3 7	3 3		7 1	4 2	4 4	4 9	4 5	4 6	4 7	3 9
Chloride mEq/l	98	95	106		89	98	95	93	93	102	66	83
Standard bicarbonate mEq/l	10 2	18 4	26 1				29 8		22 1	11 5		29 4
Base excess mEq/l			+4 0							—17 5		
Calcium mEq/l			4 3							2 4		
Inorg phosphorus mg%	6 7	5 7	4 2		16 0	11 3	6 7	9 7	6 4	13 9	16 0	7 7
Magnesium mEq/l						3 2	2 5	3 5	2 7		2 5	2 1
Serum protein g%	6 1	6 6	6 8			5 2	5 8	5 5	5 5	7 0	7 4	6 5
Interference pattern and amplitude ≥ 1 5 mV	+	+	+	+	+	+	+	+	+	+	+	+
Spontaneous fibrillation potentials	0	0	+	0	0	0	0	0	0	0	0	0
Motor unit potentials: Number	70	61	61	48	45	61	80	78	84	52	39	60
Mean duration msec	11 2	11 2	10 5	10 1	9 1	10 8	10 3	11 2	11 7	10 8	12 7	10 2
% dev from normal	+6	+6	—1	—5	—8	+9	+4	+13	+18	—4	+12	—10
% polyphasic pot	1	5	3	2	2	2	4	1	1	0	0	0
Motor nerve conduction velocity m/sec	63	64	69	68	54	56	55	52	39	48	46	63
Distal latency msec	3 0	2 7	2 1		2 9	2 8	2 9			2 2	3 3	2 9
Corrected delay msec	2 9	2 6	2 2		2 8	2 9	3 0			2 1	3 2	2 9
Total amplitude mV	9	6	14 5		24	19	24			21	20	16 5

Examination			D					D			D			D
		147	148	157	155	156	177	180	185	188	163	176	179
Repetitive stimulation % amplitude change	10/sec				± 0	—11	+10	—36	—26	+13	+ 9	— 6	—31
	30/sec				—21	—24	+ 4	—33	— 6	+ 9	+13	—31	— 6
	50/sec				—25	—26	— 9	—37	—19	— 1	+ 6	—18	—18
Post tetanic stimulation % amplitude change	10/sec				±0	+ 7	+ 2		— 6	— 8	+ 4	— 4	
	30/sec				—11	—10	±0		— 1	+ 5	+ 6	— 6	
	50/sec				— 3	— 9	— 6		—17	—12	+10	— 8	
Intramuscular temperature °C musc biceps brachii													
Muscle water g/100 g FFS musc tibialis anterior													

Examination		171	181	182	183	189	191	172	173	178	207	208	211
			D			D						D	
Age		53	53	53	53	53	53	65	65	65	15	15	15
Sex		F	F	F	F	F	F	M	M	M	M	M	M
Duration of uraemia > 1 month		yes	yes	yes	yes	yes	yes	yes	yes	yes	no	no	no
Non protein nitrogen mg%		224	247	103	138	226	107	230	265	238	257	165	125
Sodium mEq/l		126	113	116	112	121	122	130	141	136	117	134	129
Potassium mEq/l		5 3	5 0	4 1	4 9	4 3	4 0	6 9	3 3	2 6	6 2	4 5	3 9
Chloride mEq/l		80	75	83	82	77	90		106		85	95	97
Standard bicarbonate mEq/l		20 0	22 2	28 2	25 2		21 1	9 9	22 0	25 2	12 3	18 2	22 1
Base excess mEq/l		—4 0	—1 0		+3 0			—18 5	—1 0	+3 0	—15 5		—1 0
Calcium mEq/l					3 5			3 5	2 9		2 0		2 2
Inorg phosphorus mg%			10 7	4 7	7 5	14 8	8 1	5 6	7 3	7 3	10 8	14 6	10 3
Magnesium mEq/l			5 7	2 6							1 0	1 9	1 7
Serum protein g%			6 6	5 4	6 0	6 6	7 0	8 2	6 9	7 1	3 4	3 9	3 8
Interference pattern and amplitude ≥ 1 5 mV		+	+	—	+	+	—	—	—	—	+	+	+
Spontaneous fibrillation potentials		0	0	0	0	0	0	0	+	0	0	0	0
Motor	Number	38	50	73	50	51	47	65	70	39	51	47	63
unit	Mean duration, msec	10 5	11 5	13 6	11 2	11 6	11 8	12 4	11 1	12 9	11 8	10 3	10 4
poten	% dev from normal	—9	±0	+18	—3	+1	+3	+2	—9	+6	+23	+7	+8
tials	% polyphasic pot	3	2	4	2	4	2	0	3	0	2	2	0
Motor nerve conduction velocity m/sec		52	54	51	35	47	40	46	47	53	62	55	62
Distal latency msec		2 8	2 7	2 1	2 9	4 0	4 1	2 9	3 0	2 3	2 0	2 3	2 2
Corrected delay msec		2 7	2 6	2 2	3 0	4 1	4 2	2 8	2 9	2 2	2 1	2 0	2 1
Total amplitude mV		22	21	19	20	21	22	12 5		11	11	29	10 5

Examination				D			D					D
		171	181	182	183	189	191	172	173	178	207	208	211
Repetitive stimulation % amplitude change	10/sec	+15	+ 9	— 7	+13	+11	—31	—11	— 4	—22	—19	—13	+ 7
	30/sec	+16	+12	—16	+ 3	+ 6	—35	—14	— 7	—24	—21	+10	— 2
	50/sec	— 7	+ 1	—22	—19	+ 4	—40	—25	—17	—21	—20	—14	—13
Post tetanic stimulation % amplitude change	10/sec	— 3	— 7	— 2	+ 3	± 0	—13	± 0		— 9	+ 3	+10	— 5
	30/sec	— 4	+ 3	— 4	+ 6	± 0	— 3	— 5		—11	+16	+ 5	—16
	50/sec	— 1	± 0	± 0	+ 6	+ 1	—16	— 4		—11	+20	—12	— 7
Intramuscular temperature °C musc biceps brachii													
Muscle water g/100 g FFS musc tibialis anterior													

Examination	6	7	31	32	49	51	50	52	64	67	77	85
Age	65	65	63	63	44	44	34	34	53	53	21	21
Sex	F	F	F	F	M	M	F	F	M	M	F	F
Duration of uraemia > 1 month	no	no	0	0	no	no	no	no	yes	yes	no	no
Non protein nitrogen mg%	97	94	36		123	178	169	168	145	160	102	184
Sodium mEq/l	120	129	137	131	135	132	137	135	132		121	140
Potassium mEq/l	2.8	4.9	2.7	3.4	7.4	4.6	5.9	5.9	4.0		6.7	3.6
Chloride mEq/l	100	112	104	104	110	98	99	100			96	90
Standard bicarbonate mEq/l	20.7	16.7	28.7	27.5	18.2	16.3	22.1	18.7	10.4	22.1	20.9	23.2
Base excess mEq/l			+7.5	+6.0	—6.0			—6.0	—19.5	—1.0		+0.5
Calcium mEq/l	4.4		5.1		4.0		3.8	4.1			3.5	
Inorg phosphorus mg%	2.2	4.2	3.2			9.3	5.4	8.0			4.0	
Magnesium mEq/l												
Serum protein g%	5.4	5.0	7.6				5.1	6.2		6.4	5.0	6.8
Interference pattern and amplitude ≥ 1.5 mV	+	+	+	+	—	—	+	+	+	+	+	+
Spontaneous fibrillation potentials	0	0	++	0	0	0	0	0	+	0	0	+
Motor unit potentials: Number	37	63	30	20	59	53	48	43	52	56	48	49
Mean duration msec	12.1	10.5	10.9	11.7	12.4	10.7	11.6	12.1	11.1	11.8	9.9	10.5
% dev from normal	—1	—14	—10	—3	+11	—4	+7	+12	—3	+3	—2	+4
% polyphasic pot	3	3	0	0	2	0	0	0	0	2	0	4
Motor nerve conduction velocity m/sec											51	63
Distal latency msec											3.8	2.0
Corrected delay msec											3.7	2.1
Total amplitude mV											16	17

Examination		6	7	31	32	49	51	50	52	64	67	77	85
Repetitive stimulation % amplitude change	10/sec												
	30/sec												
	50/sec												
Post tetanic stimulation % amplitude change	10/sec												
	30/sec												
	50/sec												
Intramuscular temperature °C musc biceps brachii													
Muscle water g/100 g FFS musc tibialis anterior													

Examination	80	84	159	D (159/160) 160	169	D (169/170) 170	289	D (289/290) 290	310	D (310/311) 311	432	D (432/433) 433
Age	49	49	70	70	30	30	42	42	44	44	67	67
Sex	M	M	F	F	M	M	F	F	F	F	M	M
Duration of uraemia > 1 month	yes	yes	no	no	no	no	yes	yes	yes	yes	no	no
Non protein nitrogen mg%	181	144	302	164	185	119	252	95	229	172	171	108
Sodium mEq/l	119	127	146	140	130	133	120	118	116	121	133	[illegible]
Potassium mEq/l	4.1	4.2	6.0	[illegible]	6.1	4.6	4.8	3.4	6.6	4.1	3.7	3.4
Chloride mEq/l	72	85	92	94	94	99	96	93	94	97	90	97
Standard bicarbonate mEq/l	17.3	18.5	21.8	22.0	18.2	0.6	9.0	19.?	6.1	15.6		
Base excess mEq/l		—6.0								—11		
Calcium, mEq/l	9						4.3	5.6	2.7		3.0	4.7
Inorg phosphorus mg%	5.7	9.9	10.6		10.6	9.2	16.5	7.2	10.5		9.4	[illegible]
Magnesium mEq/l							1.9	1	3		2.5	2.0
Serum protein g%	5.8	5.9	6.4	6.8	7.1	9.0		5		[illegible]	6	[illegible]
Interference pattern and amplitude ≥ 1.5 mV	+	+	+	+	—	—					+	+
Spontaneous fibrillation potentials	0	0	0	0	0	0	0	0	0	0	0	0
Motor unit potentials: Number	58	51	51	63	56	67	42	52	56	59	62	89
Motor unit potentials: Mean duration msec	13.1	12.4	12.3	11.1	12.0	10.1	11.9	11.0	11.8	10.7	11.1	10.1
Motor unit potentials: % dev. from normal	+15	+9	—1	—10	+13	—5	+7	—1	+5	—4	—10	—18
Motor unit potentials: % polyphasic pot.	0	4	2	3	0	1	0	0	0	0	2	4
Motor nerve conduction velocity m/sec	55		52	50	58	56	58	61			56	58
Distal latency msec	3.0		2.9	2.2	3.0	4.1	2.4		3.0	3.2	3.3	2.9
Corrected delay msec	3.0		3.2	2.5	2.9	1.2	2.4				3.2	2.6
Total amplitude mV	25		20	15	20	9	15		11.5	15		

Examination		80	84	159 D	160 D	168 D	170 D	289 D	290 D	310 D	311 D	432 D	433 D
Repetitive stimulation % amplitude change	10/sec			+19	+ 5	—15	— 2	+ 5	+ 8	+23	+ 9	+ 5	+11
	30/sec			+ 6	— 2	— 7	± 0	—19	—21	+11	+ 8	—13	—16
	50/sec			— 8	— 9	—12	— 9	—21	— 8	+ 9	— 7	—29	—27
Post tetanic stimulation % amplitude change	10/sec				— 4	— 8	— 2	+12	— 5	+ 6	+ 7	+ 2	+34
	30/sec				— 1	— 2	— 5	+ 3	— 1	+ 1	± 0	± 0	— 7
	50/sec				± 0	+ 8	—16	+ 4	+11	+ 7	+ 2	— 6	— 8
Intramuscular temperature °C musc biceps brachii												35 6	35 9
Muscle water g/100 g FFS musc tibialis anterior												430	389

Examination	D 150	151	D 155	157	D 503	504	D 521	523	529	530	559	561
Age	63	63	35	35	62	62	34	34	40	40	65	65
Sex	M	M	M	M	M	M	F	F	F	F	F	F
Duration of uraemia > 1 month	no	no	yes	yes	no	no	yes	yes	0	0	0	0
Non protein nitrogen mg%	198	112	123	86	249	139	270	102	27	25	17[1])	19[1])
Sodium mEq/l	131	122	126	128	134	130	134	130	133	134	134	126
Potassium mEq/l	4 2	3 5	3 4	3 4	7 6	3 7	3 7	2 9	2 1	3 3	3 0	4 3
Chloride mEq/l	90	88	82	90	100	105	99	100	68	101	98	106
Standard bicarbonate mEq/l			28 9		13 1	21 5	6 0	20 5	17 0	26 6	28 5	
Base excess mEq/l			+6 0		—15 5	—3 0	—21 0	±0	+20 0	+3 0	+5 0	
Calcium mEq/l	4 3	5 8	3 5	3 6	5 1	5 8	5 0	4 0	3 6	4 0	6 9	4 4
Inorg phosphorus mg%	8 4	5 9	3 5	2 7	12 9	9 5	7 0	3 5	3 3		2 0	2 4
Magnesium mEq/l	2 0	2 1	3 5	2 8	2 3	1 6	2 8	1 8	0 5			1 9
Serum protein g%	6 2	6 8	5 2	5 1	5 9	5 5	6 5	6 6	6 5	7 6	5 6	6 6
Interference pattern and amplitude ≥ 1 5 mV	+	+	+	+	—	—	—	—	+	+	—	+
Spontaneous fibrillation potentials	0	0	0	0	0	0	0	0	0[2])	+4	+	0
Motor unit potentials: Number	66	42	79	79	36	45	50	77		49	37	69
Motor unit potentials: Mean duration msec	10 7	8 9	11 0	9 9	12 1	12 1	11 8	10 8		11 6	11 1	9 9
Motor unit potentials: % dev from normal	—12	—26	+1	—10	+1	+1	+9	±0		+5	—9	—19
Motor unit potentials: % polyphasic pot	0	7	4	5	0	9	4	4		10	8	3
Motor nerve conduction velocity m/sec	59	61	54	58	46	46	54	50	55	61	64	66
Distal latency msec	2 5		3 0	3 0		3 8	3 0	3 7	2 8	2 2	2 6	2 9
Corrected delay msec	2 2		2 7	2 9		3 7	3 1	3 9	2 7	2 3	2 4	2 8
Total amplitude mV			24	13			25 5		8	22	5 2	13 5

Examination		D 450	D 451	D 455	D 457	D 503	D 504	D 521	D 523	529	530	559	561
Repetitive stimulation % amplitude change	10/sec	+ 2	—10	—12	—16	— 3	± 0	+10	+ 6	+ 6	+53	+ 5	+ 5
	30/sec	— 2	— 5	—13	+31	—31	— 6	—14	+15	+26	+20	—31	+15
	50/sec	+19	+28	—24	+18	—52	— 6	+17	+79	+51	+20	—11	— 1
Post tetanic stimulation % amplitude change	10/sec	+ 3	+ 7	—12	— 7	—19	— 7	+ 6	± 0	— 5	— 3	+ 7	+ 7
	30/sec	—20	+ 3	—19	— 6	—18	—36	+ 7	— 7	± 0	+ 5	—23	+ 1
	50/sec	+ 5	+ 7	—10	— 5	+ 6	—28	— 3	+56	+38	— 9	—17	± 0
Intramuscular temperature °C musc biceps brachii		36 3	36 6	35 7	36 4	35 0	34 6	35 1	37 8	36 4	35 9	36 8	36 1
Muscle water g/100 g ITS musc tibialis anterior		409	373	420	393	429	368	398	362	434	385	140	461

1) Blood urea N in mg per cent not included in the statistical calculations
2) Profuse spontaneous activity

Examination	14	15	37	53	54	56	62	69	71	72	91	105	139
Age	59	72	64	24	53	57	65	69	26	41	54	70	41
Sex	M	M	M	M	M	M	F	M	M	F	F	F	M
Duration of uraemia > 1 month	yes	yes	no	no	no	no	yes	yes	yes	0	no	no	yes
Non protein nitrogen mg%	183	293	151	124	298	120	236	82	155	45	366	120	162
Sodium mEq/l	133	132	140	128	123	140		134	132	145	138	145	135
Potassium mEq/l	7 9	7 0	7 2	5 9	5 9	3 2		5 9	4 1	2 7	7 4	3 3	4 3
Chloride mEq/l	87	96	94	88	70	99		102	100	99	83	105	90
Standard bicarbonate mEq/l	14 5	7 1	20 1	25 6	20 4	26 0	15 5	19 2	17 6	33 5	13 4	10 4	18 2
Base excess mEq/l	—12 0	—22 0			—3 0		—13 0	—5 0	—7 0	+13 0	—13 5	—19 0	
Calcium mEq/l		4 1	4 7	2 1	3 6	6 1		4 2	3 2		2 5		
Inorg phosphorus mg%		12 1	10 3	11 9	8 3	5 0		3 2	8 9		15 8		12 7
Magnesium mEq/l													
Serum protein g%	6 3	6 8	7 3	4 0	4 4	6 0		4 9	5 0	4 9	5 9		6 4
Interference pattern and amplitude ≥ 1 5 mV	—	—	—	—	+	+	—	+	+	+	—	+	+
Spontaneous fibrillation potentials	0	0	0	0	0	0	0	0	0	+	0	0	0
Motor unit potentials: Number	58	27	30	37	68	66	43	68	71	52	44	68	50
Motor unit potentials: Mean duration msec	10 7	11 7	12 3	12 2	13 6	12 5	8 3	11 2	11 1	12 6	12 3	12 9	10 8
Motor unit potentials: % dev from normal	—9	—6	+2	+20	+18	+7	—32	—9	+7	+14	+6	+4	—3
Motor unit potentials: % polyphasic pot	0	0	7	0	7	3	9	6	4	2	2	0	2
Motor nerve conduction velocity m/sec					51	71		56	54	69	44	66	53
Distal latency msec						2 3		2 2	2 3	2 9		2 2	2 2
Corrected delay msec						2 1		2 3	2 2	3 0		2 3	2 1
Total amplitude mV						23		11 5	17	19		21 5	22

Examination		14	15	37	53	54	56	62	69	71	72	91	105	139
Repetitive stimulation % amplitude change	10/sec													
	30/sec													
	50/sec													
Post tetanic stimulation % amplitude change	10/sec													
	30/sec													
	50/sec													
Intramuscular temperature °C musc biceps brachii														
Muscle water *g/100 g FFS* musc tibialis anterior														

Examination	174	206	212	240	300	420	428	445	446	460	463	471
Age	41	38	48	65	62	59	46	51	35	26	47	65
Sex	F	M	F	F	M	M	M	M	F	M	F	F
Duration of uraemia > 1 month	yes	yes	no	no	no	no	yes	no	0	0	yes	no
Non protein nitrogen mg%	133	262	270	171	79	123	191	135	37	34	300	167
Sodium mEq/l	128	131	132	127	142	148	136	118	134	132	121	122
Potassium mEq/l	4.8	4.3	5.9	5.9	3.3	5.7	5.2	5.2	3.2	4.8	4.1	5.1
Chloride mEq/l	88	94	86	81	94	112	93	88	102	108	87	92
Standard bicarbonate mEq/l	22.1	13.5	13.6	19.9	29.9	16.0	17.6	18.2	25.5	22.5	13.1	
Base excess mEq/l	—1.0	—13.0	—13.0		+7.0	—9.0	—7.9	—7.8	+2.0		—12.5	
Calcium mEq/l	4.4	4.2	2.7	1.4	6.4	4.1	4.4	4.0	4.4	3.2	4.1	5.0
Inorg phosphorus mg%	8.6	13.1	13.0	15.0	2.7	4.8	8.5	7.5	3.7	4.5	12.8	14.0
Magnesium mEq/l			1.9					2.4	1.8	1.9	2.4	
Serum protein g%	6.1	6.3	4.3	5.0	5.7	5.9	6.5	5.4	6.8	5.2	6.8	6.2
Interference pattern and amplitude ≥ 1.5 mV	+	+	+	—	+	+	+	+	+	+	+	—
Spontaneous fibrillation potentials	0	0	0	0	0	0	0	0	0	0	0	0
Motor unit potentials: Number	36	53	69	31	33	50	36	55	94	43	52	61
Motor unit potentials: Mean duration msec	10.8	9.6	10.7	12.4	11.8	10.7	10.9	11.1	11.3	10.7	12.3	11.2
Motor unit potentials: % dev from normal	—3	—13	—5	+2	—2	—9	—3	—3	+4	+3	+9	—8
Motor unit potentials: % polyphasic pot	0	2	3	3	15	2	0	2	1	2	2	3
Motor nerve conduction velocity *m/sec*	60	53	49	58	42	55	52	68	65	54	45	49
Distal latency msec	2.6	2.9	3.1	3.9	3.1	3.1	3.0		2.1	2.8	2.8	3.0
Corrected delay msec	2.7	3.0	3.2	3.6	2.7	2.8	2.7		2.2	3.0	3.4	2.9
Total amplitude mV	10	14.5	10.5	11						21.5	30	12

Examination		171	206	212	240	300	170	478	445	446	460	463	471
Repetitive stimu-	10/sec	—10	+ 7	—12	— 1	+12	+ 1	3	+ 3		— 7	+11	—20
lation % amplitude	30/sec	—12	+14	+10	—16	± 0	— 6	+ 9	+21		+20	+27	— 9
change	50/sec	— 5	+16	+30	—37	— 9	— 5	+ 1	+16		+21	+19	—23
Post tetanic stimu-	10/sec	+ 1	± 0	—16	— 3	± 0	— 1	— 8	—12		± 0	+ 6	— 2
lation % amplitude	30/sec	— 4	+ 1	—19	— 8	± 0	— 1	+ 7	+ 5		+17	± 0	—11
change	50/sec	— 7	± 0	+16	—11	+ 2	± 0	+ 8	± 0		+18	+ 2	— 6
Intramuscular temperature °C													
musc. biceps brachii									35.9	36.1	37.0	36.7	35.7
Muscle water g/100 g FFS													
musc. tibialis anterior							375	389	391	391	357	375	410

Examination		473	479	506	543	550	551	552	563	566	567	568	569
Age		25	20	20	70	49	22	49	28	26	22	42	64
Sex		F	F	M	M	M	F	M	F	M	M	F	M
Duration of uraemia > 1 month		0	0	yes	yes	yes	no	no	yes	yes	yes	yes	0
Non protein nitrogen, mg%		28	32	251	223	200	132	243	249[1])	219[1])	188[1])	160[1])	31[1])
Sodium mEq/1		130	140	127	141	135	118	161	137	122	134	132	134
Potassium mEq/1		3.6	3.6	5.4	5.1	3.8	4.5	5.8	4.4	5.4	4.7	3.6	2.9
Chloride mEq/1		106	103	88	104	106	82	124	88	82	98	89	102
Standard bicarbonate mEq/1		24.5	23.4	17.5	10.7	10.6	23.5	17.2	20.5	15.5	22.7	27.5	
Base excess mEq/1		+1.0	—0.5	—7.5	—17.0	—18.5	—0.5	—9.5	—4.0	—11.0	—1.5	+4.5	
Calcium mEq/1		1.6	4.7	3.7	4.2	3.0	4.0		4.1	3.4	5.1	4.3	7.1
Inorg phosphorus mg%		3.0	3.5	9.5	5.5	7.8	8.2	17.8	11.3	10.8	8.3	6.1	2.0
Magnesium mEq/1		1.8	2.1	1.8				3.3	4.0	1.1	2.5	3.1	1.7
Serum protein g%		6.5	6.8	6.1	6.3	7.2	5.2	4.5	6.4	8.2	6.2	6.2	6.3
Interference pattern *and amplitude* ≥ 1.5 *mV*		+	+	+	—	+	+	—	+	+	+	+	+
Spontaneous fibrillation potentials		0	0	0	0	0	0	+	0	0	0	0	+
Motor	Number	43	81	58	78	45	50	34	44	38	34	43	36
unit	Mean duration, msec	11.7	10.0	11.4	13.4	12.2	11.3	11.3	11.7	13.2	9.8	10.5	14.2
poten	% dev from normal	+14	±0	+14	+8	+7	+12	—1	+11	+27	—3	—5	+18
tials	% polyphasic pot	5	0	0	3	0	12	9	2	0	12	5	8
Motor nerve conduction velocity m/sec		73	65	59	43	48	53	69	55	43	53	54	55
Distal latency msec		2.1	2.0	2.5	3.0	2.5	2.5		2.3	3.9		2.9	
Corrected delay msec		2.2	2.2	2.4	3.0	2.4	2.4		2.4	4.0		3.2	
Total amplitude mV		15		27	15	25	11.5		15.5	10		15	

Examination		473	479	506	543	550	551	552	563	566	567	568	569
Repetitive stimu	10/sec	—13	—11	+ 3	+ 8	+ 6	+35	+20	—16	± 0	+ 2	+ 2	+25
lation % amplitude	30/sec	—10	— 5	+ 3	+ 9	+17	— 1	+54	+26	+ 5	+18	— 6	— 7
change	50/sec	+57	—23	— 6	+ 7	+10	+ 5	—10	+ 9	— 3	+16	—10	—39
Post tetanic stimu	10/sec	— 3	± 0	± 0	— 2	— 1	— 3	+ 1	— 9	— 3	± 0		— 8
lation % amplitude	30/sec	—28	— 5	— 3	+ 2	+ 3	— 2	+72	+ 8	—10	—11	— 8	+ 4
change	50/sec	—14	— 3	+ 3	— 1	— 2	± 0	+37	+ 6	— 3	+10	— 6	— 4
Intramuscular temperature °C													
musc biceps brachii		36 9	36 8	36 3		36 1	36 3	37 0	35 6	36 0	35 6	36 1	36 7
Muscle water g/100 g FFS													
musc tibialis anterior		351	352	430	400	412	396	314		398	411	433	348

[1]) Blood urea N in mg cent not included in the statistical calculations

Acta Medica Scandinavica

Supplementum 492

Antibody Activity in Monoclonal Immunoglobulin G

by

OLLE ZETTERVALL

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv*, was founded in 1869 by Professor Axel Key, MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*

Acta Medica Scandinavica

publishes papers on general medicine from Denmark, Finland, Iceland, Norway, Sweden and the Netherlands. Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English French or German. Acta Medica Scandinavica is published on a non-profit basis.

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each a year) include free supplements of the current volumes During the past few years an average number of 17 supplements has been issued per annum.

Subscription Rates

Per annum=two volumes.
In Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Sw kr 120, incl. postage
Other countries Sw kr 140, incl. postage

Chief Editor

Associate Professor Birger Strandell, MD
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Biblioteksgatan 11
S-111 46 Stockholm, Sweden

Subscription and Distribution

Almqvist & Wiksell
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1, Sweden

Printers

Almqvist & Wiksells Boktryckeri AB
Box 616
S-751 25 Uppsala 1, Sweden

ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 492

FROM THE DEPARTMENT OF MEDICINE
(HEAD PROFESSOR JAN WALDENSTRÖM M D)
UNIVERSITY OF LUND ALLMÄNNA SJUKHUSET MALMÖ
AND THE DEPARTMENT OF NEUROLOGY
(HEAD PROFESSOR RAGNAR MULLER M D)
UNIVERSITY HOSPITAL, LUND SWEDEN

Antibody Activity in Monoclonal Immunoglobulin G

by

OLLE ZETTERVALL

D 1968

Supported by grants from The Swedish Cancer Society (project No 142 B 67-01 X)
the Medical Faculty of the University of Lund and by Alfred Österlunds Stiftelse

Printed in Sweden
SYDSVENSKA DAGBLADETS AKTIEBOLAG

Contents

INTRODUCTION

Humoral antibodies belong to the collection of proteins known as the immunoglobulins The immunoglobulins are defined by certain structural properties some of which are shared by the majority of the members of this family of proteins Our present concept of a basic four chain structure originates from Edelman s (1959) work on chemically produced subunits and from Porter s (1959) work on enzyme cleavage products from immunoglobulin The structure is thought to consist of 2 heavy and 2 light polypeptide chains united by covalent (disulfide) bonds and in addition by non-covalent forces Isolated heavy and light chains may be prepared and subsequently recombined to four chain molecules When the chains are prepared from antibody active immunoglobulin, the reconstituted four chain molecules characteristically display antibody activity (Olins and Edelman 1964)

Heterogeneity among the immunoglobulins is immediately conceivable considering the wide spectrum of antibody specificities that may be elaborated in each individual Immunoglobulin *i e* antibody heterogeneity is however not limited to variations in antibody specificity Thus human immunoglobulins display a large variety of electrophoretic mobilities There are further differences in antigenic configurations on the heavy chains defining classes of which the quantitatively most predominant are IgG * IgA and IgM Recently 2 more classes have been described *i e* IgD (Rowe and Fahey 1965 a b) and IgE (IgNd) (Ishizaka Ishizaka and Hornbrook 1966 Johansson and Bennich 1967) Heavy chain antigenic subclasses have been found in IgG (Grey and Kunkel 1964 Terry and Fahey 1964), in IgA (Feinstein and Franklin 1966 Kunkel and Prendergast 1966, Terry and Roberts 1966, Vaerman and Heremans 1966) and in IgM (Harboe et al 1965) Similarly antigenic differences have been recognised in the light chains and 2 main types are known, *i e* K and L (Fahey 1963, Mannik and Kunkel 1963) Genetically determined factors Gm characters are known in human IgG (Grubb 1956) and are associated with the heavy chains Other allotypic factors (Inv) (Ropartz et al 1961) are found on human light chains of type K Electrophoretic heterogeneity, classes subclasses and allotypic variants of IgG are also found in species other than man The molecular weights of the immunoglobulins vary between approx 900 000 for IgM down to 150 000 for IgG and IgA Higher molecular weights of IgA due to polymerisation are also encountered For a recent review, see Cohen and Milstein (1967) A further dimension of heterogeneity is apparent from studies on purified antibodies as a variation in the structure of the antibody combining site even in cases where a well-defined antigenic configuration has been employed This has for instance been demonstrated as a heterogeneity of binding affinities for the antigen in certain anti hapten antibodies (Eisen and Siskind 1964)

The use of homogenous or almost homogenous immunoglobulins *i e* M-components has greatly facilitated the study of immunoglobulin structure M components are not normally found but occur, often in huge quantities, in a multitude of clinical conditions malignant or not The fact that it has been possible to correlate almost all findings obtained with M components with properties of

* The terminology recommended by the World Health Organization (Bull Wld Hlth Org 30 447 1964 33 721 1965 35 953 1966) was followed

normal immunoglobulins has created the belief that M-components are to be considered as representatives of normally occurring proteins, or even that the normal immunoglobulins could be regarded as a collection of M-components (Waldenstrom 1961, Kunkel 1965) In view of this antibody active M-components as models of ordinary antibodies have been looked for

Rheumatoid factor activity may be demonstrated in certain IgM M-components (Kritzman et al 1961) and cold agglutinin activity in others (Christensen et al 1957, Fudenberg and Kunkel 1957) Anti lecithin activity in an IgM M-component was reported by Killander, Killander, Philipson and Willen (1967) and anti-alpha and beta lipoprotein activity in an M-component of IgA class by Beaumont (1967) A serum with an IgA M component and extremely elevated anti-α staphylolysin activity has been described by Mansa and Kjems (1965) Dammacco and Clausen (1966) have found an M-component giving precipitin reaction with staphylococcal and pneumococcal extracts

The presence of antibody activity in IgG M-components was inferred by Waldenstrom (1961) from the finding of a serum with such M-component and excessive antistreptolysin-O titre Besides this case (patient EN) another serum (AO) with the same characteristics was found by Waldenstrom et al (1964) in a survey of 188 sera containing M-components This collection also contained 2 sera with considerably increased anti thyroglobulin activities Other examples of a high anti streptolysin activity associated with an IgG M-component have been given by Badin and Cabau (1964) Kronvall (1967) and by Egelund Schmidt et al (1968) and more cases may be among the further 6 instances of M-component together with elevated anti streptolysin O activity briefly given in the last mentioned paper

Anti-α staphylolysin activity in an IgG M-component was described by Mansa and Kjems (1965) A similar serum (IN) was discovered by Winblad * Recently Eisen et al (1967) reported a G-myeloma globulin reacting with a hapten, i e ε 2,4 dinitrophenyl L-lysin

No uniform clinical picture seems to be associated with the presence of a high antibody activity and an IgG M component The absence of a definite history of exposure to the appropriate antigen has been explicitly stressed in some cases (Waldenstrom et al 1964) A clinical survey of the cases AO, EN and IN will be given by Hallén et al (1968)

The finding of reactivity of an M component in an antibody assay system does not necessarily prove that the globulin is to be regarded as a true antibody Since in practice a thorough history of immunisation is impossible to obtain for an individual the question whether the M-component is a true antibody or not could only be solved by study of the intrinsic properties of the protein In the final analysis this may require an elucidation of the M-component's primary structure which to date has not been obtained for any multichain immunoglobulin However, if an antibody active M-component is found not to conform with accepted functional and structural criteria for ordinary antibodies, its activity will probably represent a pathological phenomenon Conversely, each finding of a typical antibody property in the protein increases the probability that it represents an ordinary antibody globulin

In this work the IgG M-components AO, EN and IN are investigated for the following properties a) antibody activity in the proteins, b) localisation of antibody activity in their papain fragments c) formation of antibody active molecules at recombination of their polypeptide chains, and d) binding of their presumed antigens

* The Sample of serum IN used in the present investigation was the generous gift of professor S Winblad Malmö

I Materials and methods

In this Chapter are described some methods especially developed for this work, *i e* an acrylamide gel electrophoresis system suitable for analysis of heavy and light polypeptide chains, the use of a low molecular marker for identification of molecular size in gel filtration experiments, and diffusion in-gel techniques for determination of antistreptolysin O and anti-α staphylolysin activity

In addition are given standard materials and methods commonly used in the investigations of the Chapters II—V

Additional methods or minor departures from the procedures given in this Chapter are stated in each instance

MATERIALS

Patient sera AO, EN and IN are described in Chapter II The serum SE had been obtained from a patient with a history of throat infection preceeding arthralgia It had an AST titre of 4500 U/ml

Serological reagents Streptolysin O, α-staphylolysin broth and horse standard anti-α-staphylolysin serum was obtained from the National Bacteriological Laboratory Stockholm Standard human anti streptolysin-O serum was from the Danish State Serum Institute Copenhagen

Protein preparations Pooled normal human IgG, prepared by ion exchange chromatography of a Cohn fraction was obtained as a lyophilisate from Kabi AB Stockholm Sweden On immunoelectrophoresis at a protein concentration of 1 g/100 ml employing polyvalent anti human serum, only IgG could be demonstrated This preparation is designated IgG Kabi or pooled normal IgG Human immunoglobulin against staphylococci was obtained from the National Bacteriological Laboratory The preparation was purified by ion exchange chromatography (DEAE SEPHADEX A-50 (Pharmacia) 0 0175 M phosphate, pH 6 50) It consisted of pure IgG (IgG ASTA) by the same criteria as valid for IgG Kabi IgG AL and IgG EG had been prepared by ion exchange chromatography from 2 human myeloma sera and was a gift from Dr J Sjoquist, Uppsala IgG AL is of type GK and IgG EG of type GL

Human transferrin was obtained from Kabi AB and bovine serum albumin (BSA) from Armour Pharmaceutical Co Ltd, Eastbourne England

Isotopes ^{125}I was obtained from the Radiochemical Centre Amersham, England ^{131}I was obtained from the same source, or from AB Atomenergi Studsvik, Sweden The preparations were free from reducing agents Radioiodinated human serum albumin (RIHSA) was from the last mentioned manufacturer

Chemicals With the exception of sodium dodecyl sulphate (SDS) which was of Swedish Pharmacopoeia quality, all chemicals were of reagent grade, or better they were used as delivered by the manufacturer

METHODS

Analytical and preparative methods

Spectrophotometry was carried out in a Beckman DU or Beckman B spectrophotometer 1 cm quartz cuvettes were used at ultraviolet and sometimes at visible wave lengths otherwise 1 cm glass cuvettes were employed

Determination of protein concentration For solutions of purified immunoglobulins and of polypeptide chains of immunoglobulins the extinctions at 280 nm were used One and the

same extinction coefficient was assumed for these kinds of material, *i e* $E_{1\,cm}^{1\,\%}=13\ 6$, the figure given for human gamma globulin by Crumpton and Wilkinson (1963) For other types of protein solutions, and sometimes for those already described, a modification (Eggstein and Kreutz 1955, Legget Bailey 1962) of Lowry's et al (1951) method was employed Samples of IgG Kabi solutions of known protein concentrations were used as standards Determination of total serum protein is described in Chapter II

Agar gel electrophoresis was performed by Wiemes (1959) technique

Paper electrophoresis was carried out according to Laurell, Laurell & Skoog (1956) with modifications regarding staining (Zettervall et al 1966)

Horizontal starch gel electrophoresis Poulik's (1957) tris citrate buffer system was used The gels were prepared in 0 5 × 11 × 26 cm Perspex moulds samples (20 µl) soaked into 0 5 × 1 cm strips of Whatman No 3 filter paper, were inserted into the gel and electrophoresed at +4°C for 16 hr at a field strength of about 4 V/cm Amido Black B (0 1 per cent w/v) in methanol acetic acid water (5 1 5 v/v) was used for staining (10 min) of the sliced gel Solvent without dye was used to remove any excess stain

Acrylamide gel electrophoresis Maizel's (1966) method was modified in that urea was included in sample and gel buffers further, gel plates were used instead of columns The changes were introduced to facilitate dissolution of lyophilised heavy chains difficult to dissolve in detergent alone and to enable comparison of samples analysed in one and the same gel The actual procedure was as follows To make up 1000 ml of gel solution acrylamide 50 g SDS 1 g N,N bismethylene acrylamide 1 3 g and urea 480 g were dissolved in enough 0 1 M, pH 7 2 phosphate buffer to give the assigned volume This solution was still usable after several months storage in the cold Polymerisation was achieved by addition, to final concentrations, of 0 05 per cent (w/v) N,N,N ,N -tetramethylethylene diamine and 0 075 per cent ammonium persulphate After rapid mixture of the constituents, 125 ml of the solution was poured into a 0 3 × 11 × 26 cm Perspex mould A glass plate was immediately placed over as a cover, care being taken to avoid air between the glass and the gel solution After 1—24 hrs the cover was removed and the gel was ready for use The gel contains urea at approximately 8 M and SDS at about 0 1 per cent (w/v) The protein samples, lyophilised from 0 1 M formic acid were dissolved in sample buffer, made up by dissolving, in 0 1 M, pH 7 2 phosphate, urea and SDS to final concentrations of 8 M and 1 2 per cent (w/v), respectively The samples were kept at room temperature for 1—3 hr before electrophoresis This time was arbitrary The samples (10 µl) were applied on 2 5 × 10 mm Whatman No 3 filter paper strips then applied to slits in the gel Double layers of Whatman No 3 filter paper were used as bridges to the buffer troughs, filled with 0 1 M phosphate, pH 7 2 with 0 1 per cent (w/v) SDS The horisontal gel was covered with Saran film Electro phoresis was carried out at room temperature at a field strength of initially about 2 V/cm rising in 12 hrs to about 3 V/cm To visualise the protein pattern, the gel was stained without slicing, using the same procedure as for starch gels After further destaining for a few hrs in 1 per cent (v/v) acetic acid gels have been dried under stretched water permeable cellophane So treated, they are suitable for filing When radioactive proteins, such as radioiodine labelled heavy and light chains, are to be analysed, the gel is easily cut into fractions for counting

Preparative electrophoresis in Sephadex G 25 Fine Buffer of the composition 0 05 M diethyl barbiturate, 0 01 M diethylbarbituric acid and 0 05 M sodium acetate pH 8 6 was used in gel and electrode vessels The gel was prepared on a glass plate in a block of dimensions 0 3 × 10 × 25 cm The sample, made up with dry Sephadex to a thick slurry was applied

to a 0 5 × 6 cm transverse slit in the gel block Double Whatman No 3 filter papers were used as bridges to the electrode vessels Electrophoresis was carried out at +4°C in a moist chamber at a field strength of about 5 V/cm Running times are given in each separate case After electrophoresis gel fractions of approximate dimensions 0 5 × 6 cm were scraped from the plate, and protein was eluted in standard volumes of buffer Protein in the eluates was assayed with the Lowry method

Cellulose acetate electrophoresis analytical or preparative was performed as described elsewhere (Zettervall et al 1966)

Ion exchange chromatography Either DEAE Sephadex A-50 CM-Sephadex C-50 (Pharmacia AB Uppsala Sweden) or DEAE Cellulose (Serva Entwicklungslab Heidelberg, W Germany) were used as ion exchangers in column procedures Column dimensions buffers and flow rates are given in each instance In cases where elution was carried out with a gradient procedure cylindrical open beakers of equal dimensions were used as mixing chamber and limit buffer reservoirs, respectively The beakers were connected with a siphon and buffer was pumped from the mixing chamber to the column

Gel filtration employing Sephadex G 200, particle size 40—120 μ (Pharmacia) was performed in either of the following buffers tris HCl, 0 1 M pH 8 0, phosphate buffered saline (PBS) 0 075 M phosphate 0 075 M sodium chloride, pH 6 5 or 0 1 M formic acid The column length to diameter ratio was 20 1, or higher Buffer flow maintained with a peristaltic pump, was 3 cm/h or lower Columns equilibrated with tris HCl or PBS were operated in the cold whereas those with formic acid were used at room temperature Fractions were read at 280 nm and/or assayed for radioactivity

In the gel filtration experiments with Sephadex G-200-PBS riboflavin was usually included in the samples as an internal standard A knife's point of the substance (M W 356 78) was mixed with the sample which was then cleared from undissolved dye by centrifugation After completion of chromatography the visibly yellow fractions were read at 280 nm to define the peak riboflavin fraction or this could be found by direct inspection Elution volumes for protein and riboflavin peaks were then measured or calculated from flow rates and fraction numbers The index, R_{ribo} could then be calculated for each material eluted, by inserting the respective elution volumes for the peak fractions into the definition formula R_{ribo} = elution volume of peak/elution volume of riboflavin peak When dilute (1 1 saline) human serum was analysed on three columns (diameter 0 9—1 1 cm, length 90 cm) the figures for R_{ribo} were albumin 0 637—0 666 IgG 0 498—0 545, and void peak 0 329—0 356 These ranges calculated as percentages of the respective means are 4 5 8 9 and 7 8, respectively Approximatively equally narrow ranges were obtained with radioiodinated IgG isolated heavy or light chains or with papain split products from IgG The figures for the respective R_{ribo} will be given in Chapters III and IV R_{ribo} seems to be a useful characteristic of molecular size at least in connexion with the buffer and Sephadex grade employed The index facilitates comparison of results from different gel filtration experiments Gel filtration in Sephadex G 25 is described in Chapter IV

Counting of radioactivity was performed in a Packard Auto-Gamma well crystal spectrometer With the settings employed counting efficiency was about 28 per cent for ^{125}I and 18 per cent for ^{131}I In giving the results corrections have been made for decay natural background and, when the 2 isotopes were simultaneously counted for interference with the ^{125}I channel from the ^{131}I channel No significant background from ^{125}I on the ^{131}I counts was apparent Standard preparations were included to enable comparison of results obtained at different countings

Immunochemical procedures

Preparation of antisera

Immunisation of rabbits 2 volumes of antigen solution, containing 2—3 mg of protein, was emulsified with 1 volume of Freund's complete adjuvant. A rabbit was injected subcutaneously with the mixture which was dispersed over 3—4 injection sites. Rabbits were injected once every 1—2 weeks and serum was harvested after 5 months or longer.

Absorption technique To 1 volume of antiserum was added cross reacting antigen either in <1/10 vol. as solution or as lyophilisate. The mixture was incubated at 37°C for 1 hr and subsequently overnight in the cold. Any precipitate was removed by centrifugation.

Antisera The following were prepared as described above (abbreviations in brackets).

Anti IgG 2 sera were used, each proved specific as anti IgG when used in immunoelectrophoresis of normal human serum. One of the sera was absorbed with normal human light chains. When tried at different dilutions in immunodiffusion against serial dilutions of normal human serum in conjunction with specific anti IgA and anti IgM sera the absorbed serum (*anti IgG A*) proved specific against IgG, the other serum (*anti-IgG B*) was used without absorption. *Anti normal human γ-chain* (*anti-γ-chain*) the serum was absorbed with normal human light chains. No cross reaction was obtained with light chains in immunodiffusion.

Anti normal human light chain (*anti light chain*), the serum was absorbed with normal human heavy chains. No reaction with heavy chains was discernible in immunodiffusion.

Anti individual IgG, against IgG from the patient sera AO, EN and IN, (*anti IgGAO, EN and IN* respectively). These antisera are described in Chapter II. *Anti bovine serum albumin* (*anti BSA*) The serum gave one line against BSA at immunodiffusion.

Specific rabbit *anti transferrin, anti IgA, anti-IgM* sera and polyvalent *anti human rabbit serum* (*anti human*) were obtained from Behringwerke Marburg Lahn, W Germany. Specific rabbit *anti light chain type K* (*anti-K*) *and L* (*anti L*) sera were a gift from Dr I Berggård, Uppsala.

Immunodiffusion and immunoelectrophoresis was carried out according to the modified Ouchterlony (1948) and Scheidegger (1955) methods as described by Link (1967).

Single radial immunodiffusion A modification (Fahey & McKelvey 1965) of the original method of Mancini et al (1963) was used with the exception that wells of 3 mm diameter were employed.

Tube method for determination of anti-streptolysin O (*AST*) *activity* Titration was performed according to Liao's (1951) method with certain modifications. As in the original technique doubling dilutions of the sample to be titrated was reacted with a standard amount of reduced streptolysin diluted to 1 combining unit/ml. After addition of washed sheep red cells and incubation haemolysis was estimated by reading the centrifuged and suitably diluted samples at 525 nm. The percentage of haemolysis was then calculated from a standard curve relating optical density and haemolysis percentage. The dilution of the sample to give 50 per cent haemolysis was interpolated with the nomogram of Liao, relating probit of haemolysis percentage to dilution of the sample. This interpolated dilution constituted the end point of the titration.

The actual procedure at titration was as follows. Five conical glass centrifuge tubes, numbered 1 to 5, received 0.5, 0.75, 0.875, 0.938 and 0.970 ml of phosphate buffered saline (PBS), respectively from a 1 ml pipette. Of the solution to be tested 0.5, 0.25, 0.125, 0.06 and 0.030 ml was added to the respective tubes to make up 1 ml of doubling dilutions. A slight systematic departure from the appropriate dilution factor (*i.e.* 2) is evident for the step from tube No 3 to No 4. A 1 ml pipette was used for the additions to tubes No 1—3 and a 30 µl constriction pipette for No 4 and No 5. Meanwhile streptolysin broth was reduced for 30 min at room temperature by addition of 1/25 volume of

10 per cent (w/v) sodium pyrosulphite in PBS After dilution with PBS to a concentration of 1 combining U/ml, 0 5 ml of the streptolysin was added to each of the serial dilution tubes, which were then rapidly shaken The tubes were incubated for 30 min in a 37°C waterbath To each tube was then added 0 5 ml of 5 per cent (v/v) sheep red cells in PBS After rapid mixing, haemolysis was allowed to continue for 60 min at 37°C After the addition of the red cells the tubes were again gently shaken after 15 min Incubation was terminated by addition to each tube of 2 ml PBS After mixing, the tubes were centrifugated and the clear supernatants were read at 525 nm

The standard haemolysis percentage vs OD_{525} curve had been made up from known dilutions of a saponin haemolysate of 5 per cent (v/v) sheep red cells A strict proportionality was apparent between degree of haemolysis and optical density OD_{525} corresponding to 50 per cent haemolysis in the actual AST titration system was 0 345 The sheep red cell suspensions to be used in the titrations were always adjusted to give an $OD_{5\ 5}$ of 0 345±10 per cent at 50 per cent haemolysis

The appropriate dilution of streptolysin broth to give a toxin concentration of 1 combining U/ml was obtained by allowing serial dilutions of reduced broth (1 ml) react (37°C, 30 min) with a standard AST preparation (0 5 ml) containing 1 AST U/ml Standardised sheep red cell suspension (0 5 ml) was then added Incubation for haemolysis and its evaluation was carried out as for AST titrations The streptolysin dilution giving 50 per cent haemolysis was interpolated from v Krogh's (1916) equation At this dilution the streptolysin broth was considered by definition to contain 0 5 streptolysin combining U/ml *i e* 1 ml of the dilution was considered to neutralise the 0 5 AST units from the AST standard employed

The influence on the result of the AST titration of the following factors were specially investigated (i) the titre level (ii) variation of initial dilution (iii) variation due to different batches of sheep blood and streptolysin

For study of factor (i) the following experiment was carried out Dilutions of IgG Kabi in PBS the protein concentrations of which differed by a factor of 2^n, $n=1, 2, 3$, were carefully made up Each dilution was titrated 5 or 6 times Assuming a perfect serial dilution technique at titration, identical titre figures will be obtained for the different IgG Kabi solutions when the results are corrected for the variation in initial dilution Conversely, variability of the normalised titre values could be considered to reflect imperfections in the serial dilution method In the statistical treatment of the results, standard deviations are estimated from the ranges (Tippet 1925) standard errors are calculated as $\sigma/\sqrt{n}$ The results were (i) The means of the different multiple titre determinations (corrected for initial dilutions) ranged between 6 06 and 6 99 AST U/ml, the standard deviation varied between 0 08 and 0 45, and the coefficients of variation between 1 3 and 6 5 per cent The mean titre for the whole material ($n = 23$) was 6 46, standard deviation 0 36 AST U/ml coefficient of variation 5 6 per cent and the standard error was $0\ 36/\sqrt{23}=0\ 073$ AST U/ml The mean titre for the determinations with end points in the interval between tubes 3 and 4 differed by 0 53 AST U/ml from the total mean of the material a difference compatible with random variation ($p>0\ 05$) The differences obtained with the titre means from the other determinations were even lower The type (ii) error was studied by titration of 4 different dilutions of IgG Kabi, making the ratios between the protein concentrations deviate from 2^n The coefficient of variation was 10 1 per cent The variation of type (iii) was obtained from titrations of standard preparations always included at titration Determinations taken at random from the protocols of 6 standard IgG Kabi samples frozen in aliquots and used only once after thawing were employed, 3 different batches of lysin and several different batches of blood had been used in this series of determinations The coefficient of variation was 7 2 per cent

Using the highest coefficient of variation found for the type (i) error and then considering the 3 types of errors as independent the total coefficient of variation for the titration method was calculated as

$C = \sqrt{6.5^2 + 10.1^2 + 7.2^2} = 14.0$ per cent. This means that in the case 2 titre values differ by 50 per cent, or more of the higher value, the difference is probably real ($p < 0.02$)

Micro modification of the AST tube titration method When small amounts of material were to be titrated, the reaction system volume was reduced tenfold, keeping the volumetric relation between the reactants the same as for ordinary titrations The serial dilutions were made up separately with constriction pipettes and transferred to plastic Ellerman tubes Conditions for the reaction between lysin and AST as well as for haemolysis were the same as those given for the ordinary method. The tubes were tightly capped during incubations After haemolysis, 1 ml of PBS was mixed with the contents of the tubes and the degree of haemolysis evaluated after centrifugation. The precision of the method was evaluated as for the ordinary method The type (i) error was as coefficient of variation, 4.9 per cent or less For error of type (ii), the coefficient of variation was 13 8 per cent Type (iii) error 6 titrations of standard IgG Kabi preparations, whereby 2 batches of lysin and several of blood had been used, gave the coefficient of variation of 18 2 per cent The total coefficient of variation, calculated according to the principles used for the ordinary titration method, was

$\sqrt{4.9^2 + 13.8^2 + 18.2^2} = 23$ per cent.

In polyclonal material, the correlation between the results of the ordinary and micro method appeared to be linear The graphically determined regression line had the equation $\text{titre}_{\text{ordinary method}} = 1.135 \times \text{titre}_{\text{micromethod}} - 0.03$ *However this may not be valid in* monoclonal material (Chapter IV)

Diffusion in gel method for determination of AST activity For titration of AST activity in microlitre quantities of material, scanning large numbers of samples as chromatographic fractions or for study of inhibition of AST activity by antisera, a plate method was developed. In principle, AST was allowed to diffuse in sheep blood-agar containing unreduced streptolysin After reduction AST activity was visualised as zones of haemolysis inhibition, 5 ml of 3 per cent agar (Agar-Noble Difco) in PBS at 56°C was mixed with 2 ml of 5 per cent sheep red cells, 5 U of streptolysin and PBS to 10 ml The gel solution was rapidly poured on a strictly horizontal dry glass plate and the gel was allowed to set The gel was ready for use in 15 min, or it could equally well be kept in refrigerator at least 5 days without damage Holes were cut in the agar with the same technique as described for radial immunodiffusion plates Samples, usually 5 µl, were applied to the holes with the aid of Carlsberg or Marburg micropipettes Diffusion was allowed to proceed for usually 5–10 hrs in a moist chamber Streptolysin in the plate was then activated by placing double layers of filter paper (Munktell No 50) soaked in 10 per cent (w/v) sodium pyrosulphite in PBS carefully on the gel surface After 7–10 min the filter paper was removed Haemolysis was continued until lysed areas appeared clear, or nearly so, and zones of haemolysis inhibition, if present, sharply demarcated. The plate was fixed by immersion in 10 per cent neutralised formalin–0 9 per cent (w/v) sodium chloride After 12 hrs, or longer the plate was washed in distilled water for at least 1 hr It was then dried with the same technique as used for immuno-diffusion agar plates Two perpendicular diameters of the haemolysis inhibition zones were then read to the nearest 0 1 mm with a slide caliper and their means calculated When AST titres were to be determined known serial dilutions of a solution of the material to be tested were applied onto the same plate as standards AST solutions from one source could not necessarily be titrated with the aid of standard preparations of different origin since the type of relation between tube titres and zone dia

meters varied for different materials. Since by trial and error, the relation between log tube titre and zone diameter for one material, *i.e.* serum SE, was found to be approximately linear, calibration curves for all other materials were plotted with the logarithmic transformation as above.

The precision of the method was tested in the following way. Two plates, poured at the same time, were punched with 25 regularly spaced holes. Doubling dilutions of serum SE with an AST activity by the tube method of 45—2.8 U/ml were pipetted in series in the uppermost and the middle row of holes. Two other dilutions of serum SE to be tested, at 30 and 7.5 U/ml respectively were made up. For each particular hole, not occupied by calibration solution, either of the 2 test solutions had been alloted by means of random numbers and the samples were pipetted according to this scheme. 5 µl applications of calibration and test solutions were employed. For each plate, 2 sets of figures for either test solution was obtained when both sets of serial dilutions were used independently for calibration. A third set was obtained for each of the two test solutions by referring diameters to a mean standard curve, plotted with the means of the corresponding diameters from the two original calibration series. The curves were drawn as polygons. Pipetting and reading of zone diameters were performed by a person uninformed about the design of the experiment, samples had been denoted by code symbols. Figure 1 and Table 1 illustrate the procedure.

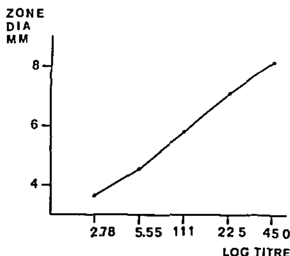


Fig. 1. Standard curve obtained by applying serial dilutions of serum SE of known AST activities (tube titre U/ml) onto a streptolysin blood agar diffusion plate. The ordinate represents the haemolysis inhibition zone diameters, the abscissa log tube titre. Duplicate standard curves were obtained from the same plate and a mean standard curve was plotted from the means of the respective zone diameters.

The calibration curves were approximately linear in the region of 5.6—45 AST U/ml (zone diameter about 4.5—8.3 mm) or slightly convex towards the axis of the zone diameters (Fig. 1). For lower AST values the curve is probably concave. Using the mean standard curve and collecting the results from the 2 plates, the findings were as follows.

The coefficients of variation of the 4 sets of measurements, calculated from the ranges, varied between 7.7 and 10.6 per cent. The differences of the 4 means and the respective true values ranged between −4.8 and 15.2,

Table 1. *Multiple determinations of AST activity (U/ml) in a dilution of serum SE.*
Aliquots of the same material were applied onto the plate used for the experiment of Fig. 1. AST titres were obtained from zone diameters referring to the mean standard curve. The Table gives the found values (v), the differences between the found values and the true value ($v-t$) plus the differences as percentages of the true values $\frac{(v-t)\times 100}{t}$. t was 7.50 AST U/ml.

v AST U/ml	7.31	7.53	6.77	7.14	8.10	7.73	6.61	7.53	7.87
$v-t$ AST U/ml	−0.19	0.03	−0.73	−0.36	0.60	0.23	−0.89	0.03	0.37
$\frac{(v-t)\times 100}{t}$	−2.53	0.40	−9.73	−4.80	8.00	3.07	−11.87	0.40	4.93

when calculated as percentages of the corresponding true values. All titre values were recalculated as differences from the true values and expressed as percentages of these, and the material was statistically treated by the analysis of variance method. No obvious difference was found between the 2 titre level groups at the 5 per cent significance level. The mean was 4.35, standard deviation 11.7 and the standard error 2.14 per cent of the true value ($n = 30$). Thus, if judged from this experiment, a titre value obtained at a single determination does not probably deviate from the true value by more than $4.35 + 2.4 \times 11.7 = 33$ per cent ($p<0.02$).

If the individual calibration curves are used, the coefficients of variation and the standard errors are approximately the same as obtained above. However, the variation of the differences of the means and the true values may be greater, up to 21 per cent of the true values.

Tube method for determination of anti α staphylolysin (ASTA) activity. Titration was performed with α staphylolysin broth diluted to 0.1 combining U/ml. The same dilution buffer (PBS) and volumes of buffer, red cell suspension, toxin dilution and antibody solution as for the AST titration system was used. Conditions for reaction between toxin and antibody (37°C, 30 min.) were also the same for the two titration methods. However, a 2 per cent rabbit red cell suspension in PBS was used and haemolysis was allowed to develop for 60 min at 37°C and subsequently for 3 hrs in the cold. After centrifugation, haemolysis was evaluated without previous dilution of the tube contents. At 50 per cent haemolysis, $OD_{5.5}$ from the standard red cell suspension amounted to 0.495, and the red cell suspensions used in the titrations were made up to give this figure within 10 per cent at the same degree of haemolysis, due allowance for dilutions being taken.

For standardisation of staphylolysin, closely spaced dilutions (1 ml) of such was allowed to react with standard horse ASTA serum (0.5 ml) diluted to 0.1 international U/ml. Reaction time was 0.5 hr at temperature 37°C. After addition of 0.5 ml of standard rabbit red cell suspension, haemolysis and assay of haemolysis were carried out as described above. The toxin dilution resulting in 50 per cent haemolysis was interpolated with v. Krogh's equation. This degree of haemolysis was used to define the end point as in the actual titrations of ASTA activity.

The precision of the ASTA titration method was determined according to the same principles as used for the AST tube titration method. The results were: type (i) error, standard horse ASTA serum used, means of the multiple values for each dilution ranged between 31.2 and 32.1 ASTA U/ml (correct figure by definition 32.0 U/ml, the serum had been used for standardisation of the toxin employed). The standard deviation varied between 0.52 and 2.2 U/ml and the limits for the coefficient of variation were 1.6 and 7.0 per cent. The mean titre of the whole material was 31.7, the standard deviation 1.41 and the standard error 0.29 ASTA U/ml ($n = 24$), the coefficient of variation was 4.5 per cent. The means from the various tube interstices did not significantly differ from the total mean. Type (ii) error: titrations of 4 differing dilutions of standard horse ASTA serum: coefficient of variation 11.1 per cent. Type (iii) error: 5 determinations on IgG ASTA at different occasions using 2 batches of lysin and several different batches of blood: coefficient of variation 15.5 per cent. The total coefficient of variation, calculated as was the case for the AST tube titration method thus amounted to $C=\sqrt{7.0^2+11.1^2+15.5^2}=20.3$ per cent. Since the upper limit for the coefficient of variation of type (i) has been used the actual precision is probably better. Considering the figure obtained it is infered, that a difference between 2 titre figures, obtained at different occasions, and amounting to 70 per cent or more of the higher value is probably real ($p<0.02$).

Diffusion in gel method for determination of ASTA activity. The method was used for similar purposes as was the AST plate method

ASTA active material was allowed to diffuse in rabbit blood agar plates after which staphylolysin was diffused into the system and neutralised around the points of application of samples The plates were made up by pouring 10 ml of gel solution at 56°C into plane glass Petri dishes of diameter 9 cm. The gel, made up with PBS, contained (final concentrations) agar (Agar Noble Difco) 1 5 per cent and washed rabbit red cells 1 per cent The gel was allowed to set for 15 min in a moist chamber Plates were then ready for use, or they could be stored in a moist chamber at +4°C for at least 5 days before utilisation Samples usually of 10 µl volume, were applied in wells of diameter 3 mm cut with the technique described for AST plates The plate was transferred to a humid atmosphere and diffusion was allowed to continue at room temperature for 5—10 hr Staphylolysin (15 ml) diluted with PBS to 0 1 combining U/ml was then poured on the gel layer The plate was observed intermittently for about 1 5 hr 4—5 times during that period the plate was gently rotated for a few seconds to induce mixing of the lysin, otherwise it was left strictly horizontal in a moist chamber at room temperature When zones of haemolysis inhibition, if any, were readily discernible the lysin solution was poured out from the dish and substituted with 10 per cent formalin—0 9 per cent saline After 12 hrs or more formalin treatment the plate was washed for at least 1 hr in distilled water The agar layer was loosened from the Petri dish transferred to a glass plate and dried Zone diameters were then measured as described for the sheep red cell-streptolysin plates Reading was best performed at indirect illumination with the agar surface pressed against a white background A certain error is introduced at reading zone diameters since the outline of the zones is somewhat diffuse

The precision of the method was investigated along the same principles used for the AST plate method, *i e* with 2 plates, each with 2 equal sets of known serial dilutions for calibration and 2 different known test solutions randomly spread over the wells Calibration was done with doubling dilutions of IgG ASTA, the ASTA titres of which differed between 5 89 and 0 37 U/ml For each inhibition zone perpendicular diameters were measured and their means calculated The 2 test solutions had the titres of 3 93 and 0 98 U/ml 10 µl quantities were pipetted of all solutions The standard curves (zone diameters against log tube titre) appeared sigmoid. For studying the precision of the method the mean standard curves of the 2 plates were used They were obtained by plotting against log tube titres the means of the respective diameters from the 2 calibration curves on the same plate The statistical treatment of the data was the same as that used for the AST plate method For both plates together the coefficients of variation of the 4 sets of values were 10—45 per cent and the differences between means and true values in percentage of the latter 3 7—25 per cent For the whole material the mean of differences between found values and true values was 20 2 standard deviation 36 and standard error 7 2 per cent of the true value ($n = 28$)

Miscellaneous

Storage of proteins was usually at —15 to —20°C However some batches of the standard anti-streptolysin-O and anti-α-staphylolysin sera were kept at +5°C, others were frozen in aliquots and used only once after thawing Heavy and light chains in 0 1 M formic acid were stored at +5°C.

Concentration of protein solutions was either performed by ultrafiltration in collodion bags (Membranfiltergesellschaft Göttingen, Germany) at +4°C, or by lyophilisation

II Serological, immuno- and physico-chemical characterisation of the sera AO, EN and IN

Some features of the sera AO and EN have been described in earlier publications (Waldenstrom 1961, Waldenstrom et al 1964, Zettervall et al 1966) It was shown that the excessive AST activity of these sera was concentrated to the electrophoretic gamma-globulin region Further it was found, that inside this region the antibody property appeared to be condensed in the area of the M-component In fact, a nearly quantitative recovery of serological activity was found at preparative cellulose acetate electrophoresis in the fraction corresponding to the M-component Adjacent fractions, which by definition contain background IgG, were practically inactive IgG, prepared from the sera by ion exchange chromatography, and shown to be rich in M-component by electrophoretic criteria, was highly AST active In the case of serum AO, one chromatographic fraction contained mostly background IgG and little myeloma globulin, the specific AST activity of this fraction was considerably lower than that of the M-component rich preparations This finding suggested, that background IgG from serum AO is endowed with a lower specific AST activity than is the case for the corresponding M-component In this first series of experiments, such information was not obtained for background IgG from the serum EN

In later reports (Zettervall 1967 a and b) it was given that background gamma globulin of the sera AO and EN showed negligible, or no antibody activity and this was also stated in the case of the ASTA active serum IN Further, the electrophoretic distribution of the ASTA activity in serum IN displayed a maximum in the M component region IgG preparations from serum IN, containing mostly M component material, proved highly ASTA active

The present Chapter provides more detailed account on the results scetched in the preceeding paragraph and also on some other investigations, aiming at identification and characterisation of the antibody active principle in the sera, AO, EN and IN

MATERIALS AND METHODS

Serum IN had been obtained from the patient about 8 months before the start of the experiments

Serum AO, 2 sera, designated serum AO A and B, respectively, were used Serum AO A had been obtained from a bleeding about 4 years before the experiments, whereas serum AO B was of unknown date, it had probably been stored for at least 2 5 years before use

Serum EN, 2 sera, EN A and B, were used in the first experiments about 1 5 years after the bleeding They were obtained from the patient at 5 day intervals A third serum (EN C) was first titrated 10 months after its collection The sera may have been accidently thawed

Antisera The following antisera, already described in Chapter I, were used specific *anti-IgA* and *anti IgM*, the 2 specific *anti-IgG* sera *A* and *B*, specific *anti-K* and *anti-L*, specific *anti transferrin* and specific *anti-BSA* Also used were the sera *anti-IgG AO*, *anti-IgG EN* and *anti-IgG IN*, these sera were absorbed with a mixture of IgG from 2 G myeloma sera of light chain type K and L, respectively, 10 mg of each lyophilised IgG preparation/ml of antiserum was used At immunodiffusion absorbed anti-IgG AO, anti IgG EN and anti-IgG IN still gave precipitin reactions against the respective M components, no precipitation was noted against normal IgG (Kabi)

Precipitation of lipoproteins from normal rabbit serum and rabbit anti sera was performed according to Burstein and Samaille (1958)

Inhibition of AST and ASTA activity by antisera was studied by means of the blood agar diffusion techniques, described in Chapter I Equal volumes of suitable dilutions (0 15 M sodium chloride) of the sera AO EN or IN and antisera or normal rabbit serum were mixed after which the strepto- or staphylolysin inhibitive power of the mixtures was assayed with the respective plate titration methods as given in Chapter I When the anti transferrin or the anti BSA sera were used, the patient serum dilutions had been mixed with BSA or transferrin to a concentration equal to or exceeding that of the M component When saline solutions containing transferrin or BSA at this concentration (*i e* 0 005 g/100 ml) were mixed with equal volumes of anti transferrin or anti-BSA sera, respectively, and the mixtures tested against the respective antisera and antigens in immunodiffusion experiments, antibody excess was apparent The antisera used in the inhibition experiments were, besides anti transferrin and anti BSA, anti IgG A and B, anti K and anti I In mixtures of patient sera and the antisera anti-IgG A, anti-IgG B and anti K, similar to the mixtures used in the plate experiments, antibody excess was demonstrated at immunodiffusion The same was true for the mixtures of anti L and the sera EN and IN, with serum AO as antigen and antibody from anti L these were approximatively at equivalence The antisera had been delipidised before use Without this treatment, several antisera showed AST activity This was negligible after the dextran precipitation The anti transferrin serum could not be used in experiments with ASTA material since this antiserum inhibited staphylolysin Controls employed were saline serial dilutions (saline) of patient sera saline solutions of BSA or transferrin, rabbit sera diluted 1 1 with saline The control preparations applied to the plates had been made to contain dextran sulphate and calcium chloride at the same concentrations as in the mixtures to be tested

Immunochemical procedures Immunoelectrophoresis of the sera AO, EN and IN, employing anti-IgG A, anti-K and anti L sera was used for classification of the M components

The content of light chain antigenic determinants K and L in IgG preparations was assayed in immunodiffusion experiments Serial dilutions of the preparations were tested against anti K and anti L sera and the highest dilutions giving visible precipitates were noted Knowing initial protein concentrations in the samples the protein concentrations corresponding to these highest dilutions were calculated and will be referred to as final concentrations For each protein preparation a K/L ratio could then be determined from the definition formula, K/L ratio = (final concentration against anti-L)/(final concentration against anti K)

IgG content of samples was measured with the single radial immunodiffusion technique, using a suitable dilution of anti IgG A serum in the plate Serial dilutions of IgG freshly obtained from the peak fraction at gel filtration (Sephadex G 200 0 1 M tris, pH 8 0) of IgG from pooled normal human serum, was employed for calibration M component in chromatographic fractions was determined with the same technique by use of the respective rabbit sera anti-IgGAO, EN and IN, absorbed as described above M component rich preparations were used as standard solutions

Determination of Gm groups in sera and preparations of M components was performed by the Institute of Medical Microbiology, University of Lund

Determination of serum total protein concentration was carried out according to the Lowry method The figures found based on calibration of the assay system with IgG, were multiplied with a correction factor This was found in experiments with mixtures of albumin and IgG (Kabi) with ratios of the two proteins equal to those found for the sera at electrophoresis The protein content of concentrated IgG solutions was determined by the ordinary Lowry method The values were expressed as optical densities (OD_{280})

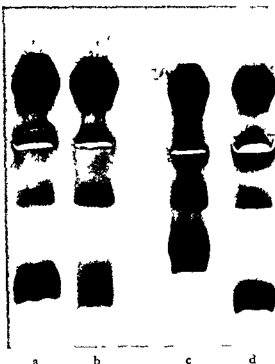


Fig 1 Agar gel electrophoresis of a) serum IN b) normal human serum, c) serum AO A d) serum EN A The anode is at the top of the figur

RESULTS

Case IN

Electrophoretic and antigenic characteristics of serum and M component

Agar gel electrophoresis of serum IN is illustrated in Fig 1 a, and of simultaneously analysed normal serum in Fig 1 b Patient serum is seen to contain an M component of γ_2-mobility, background gamma globulin is scanty and this is especially apparent when the pattern is compared with that of the gamma globulin in the normal serum

Paper electrophoresis of serum IN gave the figures of 2 6 and 0 33g/100 ml for the concentration of M component and background IgG, respectively

Starch gel electrophoresis of serum IN and of purified M component showed heterogeneity of the M component in both types of samples The two patterns appeared similar at least 3 closely spaced protein bands were discernible in each of the patterns The spacing of the bands and their position in the gel was the same for both types of material However, the relative intensities of the bands differed in that more material seemed to be distributed at the cathodic end of the pattern from the purified M component than was the case for that from the serum sample No protein fractions strictly outside the M component region were observed for the purified material

Immunoelectrophoresis of serum IN classified the M component as being of type GL

The Gm type of serum IN was Gm (1, 2, 4, 5) and that of the M component was Gm (1,2—4,—5)

Characterisation of the serum ASTA activity

Serum ASTA titre was first determined about 8 months after collection of the sample When the initial dilution of serum at titration was 1 15,000 the figures found in duplicate determinations were 3400 and 3600, mean 3500 ASTA U/ml With initial dilution 1 10,000 the titres obtained in the same experiment were 3000 and 2600, mean 2800 ASTA U/ml Heating at 56°C for 30 min did not appreciably change the titre Ageing considerably affected the serum titre Thus titres of about 700 ASTA U/ml were obtained in several aliquots of the serum when titrated about 1 year after the initial experiments, *i e* 1 5 years after collection

Electrophoretic distribution of the serum ASTA activity was first studied by preparative cellulose acetate electrophoresis of serum IN Corresponding to the albumin, α_1, α_2 β and γ regions as seen on the stained guide strips, fractions were cut and eluted in a standard volume of phosphate buffered saline (PBS) containing 0 1 per cent (w/v) BSA ASTA activity was only found in the fraction corresponding to the gamma globulin region The serum was further investigated by preparative electrophoresis in Sephadex G-25 (Fig 2) 0 5 ml of serum was fractionated for 13 hrs and protein was eluted with PBS Definite ASTA activity was localised in the fractions 20—24 There was a striking parallelism between titres and protein content Both

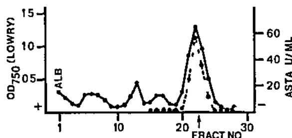


Fig 2 Preparative electrophoresis of serum IN in Sephadex G 25 block The arrow denotes the point of application of the serum The solid line gives the optical density at 750 nm at protein determination according to Lowry The broken line signifies the ASTA titre (U/ml) of the fractions titrated *i e* 15—28 No ASTA activity was found in the fractions 15—19 and 25—28 *Alb* signifies the position of the albumin fraction

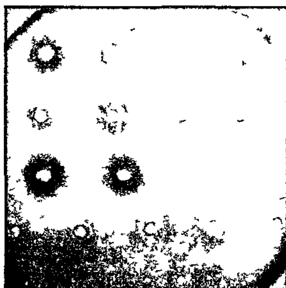


Fig 3 Inhibition of serum IN ASTA activity by anti sera From left to right Row 1 serum IN 1 1200 1 3600 1 7200 and 1 600+BSA+NaCl Row 2 serum IN 1 600+anti L 1 600+anti K 1 600+anti IgG A and 1 600+anti IgG B Row 3 serum IN 1 600+BSA+anti BSA 1 600+normal rabbit serum Row 4 anti L anti K anti IgG A anti IgG B anti BSA all mixed with NaCl Row 5 normal rabbit serum+NaCl and BSA+NaCl+dextran sulphate+CaCl

achieved their maxima for fraction 22 This eluate, concentrated to a protein content of 0 73 g/100 ml was analysed by agar gel electrophoresis and by immunodiffusion against anti-IgA and anti IgM sera Only M component was discernible in the electrophoretic pattern and no reaction with the antisera was noted Since, for instance fraction 19 gave precipitates against these antisera when analysed at a protein concentration of 0 051 g/100 ml fraction 22 could not contain more than 0 051/0 73 = 1/14 of its protein as IgA or IgM The specific ASTA activity of the protein in fraction 22 was calculated to 52 ASTA U/OD_{280}/ml The serum titre determined at the start of the preparative electrophoresis was 1480 ASTA U/ml Calculations based on the definite titres obtained and the volumes of the eluates gave the figure of 96 per cent for recovery of the serum ASTA activity in the eluates In these calculations the void volume of the Sephadex fractions had been taken in regard calculated as 1/3 of the gel volume determined at centrifugation of the fractions in graded tubes The void volume thus found amounted to 6 per cent of the buffer volume used for elution Taking the figure for the concentration of M component in serum IN from the paper electrophoretic determination already given *i e* 2 6 g/100 ml, in conjunction with the specific activity of protein in fraction 22, *i e* 52 ASTA U/OD_{280}/ml leads to the estimate of the serum titre of 1839 ASTA U/ml The concentration of M component in the serum calculated from the protein content of the M component peak was 2 5 g/100 ml (100 per cent recovery assumed)

Inhibition of serum IN ASTA activity by antisera (Fig 3) Serum IN at a dilution of 1 600 was tested BSA anti BSA was used for the unspecific precipitate control When normal rabbit serum or anti K was added to serum IN no decrease in the diameter of the haemolysis inhibition zones was apparent in comparison with the appropriate control preparations A slight decrease in zone diameter was sometimes obtained when anti BSA serum was added to the mixture of BSA and serum IN A nearly complete extinction of the haemolysis inhibition zone was noted with anti IgG

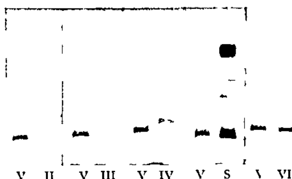


Fig 4 Agar gel electrophoresis of the chromatographic fractions IgG IN II—VI from serum IN and of the diluted (1 4) serum Roman numerals indicate the fractions and S the serum The anode is at the top of the figure

A and anti IgG B A marked inhibition was given by the anti-L serum This inhibition was always greater than that from BSA-anti BSA In addition to the lysis outside the inhibition zones there was also lysis around the central wells In the cases the inhibition zone diameter was decreased also the central lysis was impaired BSA, normal rabbit serum, the rabbit antisera and saline, gave no significant haemolysis inhibition

Preparation, characterisation and specific ASTA activities of IgG from serum IN

IgG fractions containing various amounts of background gamma globulin and M-component were obtained by ion exchange chromatography 15 ml of serum, dialysed against 0 0175 M, pH 6 50 phosphate, was chromatographed in DEAE-Sephadex A 50 equilibrated with the same buffer The buffer was also used for elution The protein emerged as one peak The major part of the eluate was fractionated in CM Sephadex C-50 "Fine equilibrated and initially eluted, with the mentioned phosphate buffer Subsequently the eluent was changed to 0 0175 M phosphate — 0 02 M sodium chloride, pH 6 40 0 0175 M phosphate —0 03 M sodium chloride pH 6 46, and from this buffer in a gradient procedure towards 0 5 M sodium chloride as limit buffer The fractions obtained during the stepwise and in the first part of the gradient elution procedures contained little protein Pools of fractions were taken from this part of the chromatogram and are designated as IgG IN I—IV, where numbering denotes order of elution Later during the gradient elution a major protein peak emerged This material was collected in 2 pools of which IgG IN V contained most of the protein IgG IN VI which was taken from the descending part of the peak was poor in protein Flow rate was approximately 30 ml/hr in both the DEAE- and the CM Sephadex chromatography and column dimensions were 2×20 cm

If necessary the pools were concentrated by ultrafiltration for the analyses to be described below

Agar gel electrophoresis (Fig 4) was performed on aliquots of the pools, dialysed against distilled water and further concentrated by lyophilisation However, IgG IN I contained too little protein and was not analysed The dried protein was dissolved in PBS so as to give solutions of the calculated protein concentration of 0 35 g/100 ml Included in the analysis was serum IN diluted 1 4 with PBS IgG IN V contained mostly M-component as shown by homogeneity of the protein and the coincidence of its electrophoretic mobility with that of the M component from the serum sample IgG II—IV contained heterogenous protein mostly migrating at the anodic side of the M component IgG VI gave the picture of a band with the approximate mobility of the M component, in addition a diffuse portion extending in the direction of the cathode was evident

Immunochemical analysis of the fractions IgG IN I—VI was carried out with immunodiffusion against anti K and anti L, and for IgG I, II, III and IV in addition with anti-IgA and anti IgM sera Further, several fractions were analysed by single radial immunodiffusion employing anti-IgG A and absorbed anti-IgG IN sera respectively

Immunodiffusion of IgG IN V at a concentration of 0 68 g/100 ml against anti IgA and anti IgM sera gave no precipitates, which,

however, was the case when normal human serum at 1 10 dilution was similarly investigated No reaction was seen with IgG IN I II and III at the concentrations (g/100 ml) 0 04, 0 07 and 0 18, respectively

The L/K ratios are given in Table 1 Also shown are the results of the single radial immunodiffusion experiments with anti-IgG A and absorbed anti-IgG IN serum given as *weight per cent of IgG based on the sample's* total protein With anti IgG A serum M-component evidently gives spuriously high values as seen for IgG IN V Using absorbed anti-IgG IN serum in the plate the figures for contamination with M component are probably overestimated since admixture of polyclonal IgG (Kabi) to IgG IN V lead to an increase of precipitin ring diameters compared to that from pure IgG IN V No ring was obtained with IgG Kabi alone or with IgG IN I and II

Specific ASTA activities of protein in the fractions IgG IN I—VI as calculated from titre and protein concentration figures for the samples are found in Table 1 The unfractionated serum IN had the titre 1200 ASTA U/ml which corresponds to a calculated specific ASTA activity of the M-component in serum of 34 ASTA U/OD_{280}/ml For the IgG fractions there is evident a rough positive correlation between specific ASTA activity on one hand and L/K ratio, as well as percentage figures from the single radial immunodiffusion experiments on the other The maximum specific activity (17 ASTA U/OD_{280}/ml) was obtained for fraction IgG IN V which also gave the maximum figures for the L/K ratio and for the percentages obtained from both types of single radial immunodiffusion experiments Since the anti-K serum detected type GK immunoglobulin in IgG Kabi, IgG IN II and III at about 0 01 g/100 ml but not in IgG V at 0 17 g/100 ml, the content of background IgG in IgG IN V could be estimated to <0 01/0 17 = 5 9 per cent On the other hand, considering the single radial immunodiffusion *data for IgG IN II it was calculated that* practically 100 per cent of the total protein content of this fraction consisted of non M-component IgG Thus the figure for specific ASTA activity of IgG IN II material, <1 5 ASTA U/OD_{280}/ml is practically the same as could be calculated for the background IgG in the sample In the calculation, the figure for the IgG content of fraction IgG IN II as obtained with the anti-IgG A serum has been subtracted with 2 times the value for the fraction s M-component content, as found with the anti IgG IN serum (The anti-IgG A serum overestimated M-component by 100 per cent as seen with IgG IN V) Similar calculations for IgG IN III gave at least 78 per cent background IgG of the total protein and a specific activity of that IgG of at most 1 7 ASTA U/OD_{280}/ml.

Case AO

Electrophoretic and antigenic characterisation of serum and M-component

Agar gel electrophoresis of serum AO A demonstrated the presence of a M-component of γ_1 mobility, background IgG of relatively low concentration was seen at the cathodic side of the band protein (Fig 1 c)

Table 1 *Immunochemical and serological characteristics of IgG fractions from serum IN*

Material IgG IN	I	II	III	IV	V	VI
L/K	<0 50	0 25	0 50	1 0	>32	2.0
IgG % w/w	46	105	110		·00	175
M-component % w/w	<7	<3 5	16	50	100	50
Specific ASTA activity U/OD_{280}/ml	<·	<1 5	<1 3	6 6	17	9 1

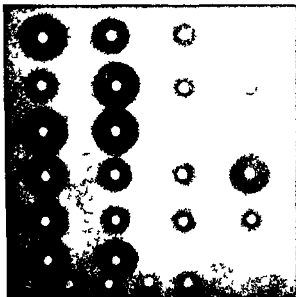


Fig 5 Inhibition of a) serum AO and b) serum EN AST activity by antisera From left to right a) Row 1 serum AO 1 2000 1 6000 1 18 000 and 1 1000+transferrin+NaCl Row 2 serum AO 1 1000+anti L 1 1000+anti K 1 1000+anti IgG A and 1 1000+anti IgG B row 3 serum AO 1 1000+normal rabbit serum 1 1000+transferrin+anti transferrin
b) Row 4 serum EN 1 2000 1 6000 1 18 000 1 1000+transferrin+NaCl Row 5 serum EN 1 1000+anti L 1 1000+anti K, 1 1000+anti IgG A and 1 1000+anti IgG B row 6 serum EN 1 1000+normal rabbit serum 1 1000+transferrin+anti transferrin Row 7 anti L anti K anti IgG A anti transferrin normal rabbit serum NaCl+dextran sulphate+$CaCl_2$ all mixed with NaCl and transferrin +NaCl+dextran sulphate+$CaCl_2$ Anti IgG B+NaCl gave no significant inhibition of haemolysis when tried in another experiment

Paper electrophoretic quantitation of M component and background gamma globulin in serum AO A gave the figures of 5 0 and 0 13 g/100 ml, respectively

Starch gel electrophoresis of serum AO together with purified M-component demonstrated heterogeneity within the myeloma globulin both in the serum and in the purified material At least 3 bands in close proximity to each other and with the same characteristic spacing and position in the gel were observed in both materials In addition the IgG preparation gave rise to a weak and more cathodic band, not seen in the serum pattern

Immunoelectrophoresis of serum AO A with anti K, anti L and anti-IgG A sera classified the M-component as a GL immunoglobulin *The Gm type of serum and M component* was Gm (1, −2, 4, 5) and Gm (1, −2, −4 −5), respectively

Characterisation of the serum AST activity

Serum AST titre Serum A was titrated in duplicate giving the figures in AST U/ml of 252,000 and 363,000, mean 308,000 Serum AO B gave the titre of 230,000 after a first thawing Thousandfold dilutions of this serum in PBS were stored frozen When titrated 5 months later, the titre obtained was 240,000 AST U/ml of undiluted serum When serum B was dialysed in the cold for 30 hr against 0 0175 M, pH 6 5 phosphate the titre dropped to 100,000 AST U/ml The change in protein concentration and volume of the serum at dialysis was insignificant

Serum AO A was heated at 56°C for 30 min and titrated in duplicate A considerable drop in titre from 308,000 AST U/ml for untreated serum to 59,000 and 73,000, mean 66 000 AST U/ml was noted

Inhibition of serum AO B AST activity by antisera (Fig 5) The serum was used at 1 dilution of 1 1000 Transferrin anti transferrin was used for the unspecific precipitation control Almost complete suppression of the haemolysis inhibition zone on the blood agar streptolysin plate was obtained with the anti IgG A and B sera Partial suppression was obvious with the anti-L serum No inhibition was discernible with the normal rabbit the anti K or the anti transferrin sera The controls without patient serum, *i e* normal rabbit serum, the antisera, transferrin and saline gave no significant inhibition of haemolysis

Preparation, characterisation and specific AST activities of IgG from serum AO A

Approximately 15 ml of serum AO A,

dialysed against 0 0175 M, pH 6 50 phosphate, was chromatographed on a 2×22 cm column of DEAE Sephadex, equilibrated and eluted with the same buffer Protein emerged in one peak The eluate was saved This preparation is referred to as IgG AO I Serum proteins, remaining on the column, were eluted with 0 175 M phosphate, pH 6 50 This eluate was concentrated equilibrated with 0 0175 M pH 6 50 phosphate and applied on a 2×22 cm column of *DEAE Cellulose (Serva)* equilibrated with such buffer The same buffer was used as eluent flow rate in both cromatographies was approx 35ml/hr Protein (IgG AO II) was eluted in one peak The material retained on the column was discarded IgG AO I and II were analysed as described below If necessary, the fractions were concentrated by ultrafiltration

Agar gel electrophoresis of IgG AO I and II was carried out at a protein concentration of 0 35 g/100 ml serum AO A diluted 1 4 with PBS was also analysed The IgG samples had been made up to the correct protein concentration by addition of 3 volumes of PBS As seen from Fig 6, IgG AO I contained electrophoretically heterogeneus protein migrating mostly at the cathodic side of the obvious M-component in IgG AO II In IgG AO II a faint protein band was found at the anodal side of the predominating M component

Immunochemical analysis of IgG AO I and II was carried out with the same technique as used for characterisation of IgG IN However, absorbed anti IgG AO serum was used in one of the single radial immunodiffusion experiments When analysed at the protein concentrations of 0 7 and 0 55 g/100 ml against anti IgA and anti IgM sera IgG AO I and II respectively gave no precipitates The 2 antisera gave precipitates against normal human serum diluted 1 5

AST titration of IgG AO I and II gave the specific AST activity figures of <1 3 and 1400 AST U/OD $_{280}$/ml respectively The L/K ratios and the results of the single radial immunodiffusion experiments as well as the specific activities found are listed in Table 2

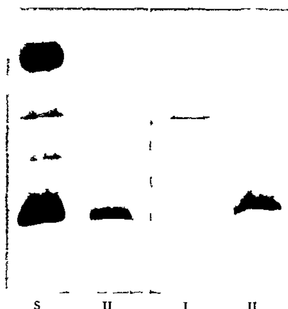


Fig 6 Agar gel electrophoresis of the chromatographic fractions IgG AO I and II from serum AO and diluted (1 4) serum Roman numerals indicate the fractions and S the serum. The anode is at the top of the figure

Judging from the single radial immunodiffusion data IgG AO I consisted of practically pure background IgG, which agreed with the electrophoretic picture of the protein and the absence of reaction against anti IgA and anti IgM sera in immunodiffusion Thus the low specific AST activity for IgG I material *i.e* <1 3 AST U/OD$_{280}$/ml was also considered to apply to the serum AO A background IgG in the sample The results of the immunodiffusion experiments with the anti IgA and anti IgM as well as the electrophoretic findings demonstrated only M component in IgG AO II

Table 2 *Immunochemical and serological characteristics of IgG from serum AO*

Material IgG AO	I	II
L/K	0 25	8 0
IgG % w/w	114	34
M-component % w/w	<5	100
Specific AST activity U OD$_{280}$ ml	<1 3	1400

besides the quantitatively unimportant protein fraction seen at electrophoresis Calculating from the final concentrations at immunodiffusion of both IgG preparations against anti K serum, the contamination of IgG AO II with background IgG was estimated as 0 009/0 08 = 11 per cent Hence most of the protein of IgG AO II seemed to consist of M-component The figure found for the specific activity of IgG AO II, *i e* 1400 AST U/OD_{280}/ml based on the total protein concentration was thus approximately the same as could formally be assigned to the M component Calculations based on the serum AST titre (308,000 AST U/ml) and the concentration of M-component in the serum (5 0 g/100 ml) lead to a formal specific AST activity of 4500 AST U/OD_{280}/ml for the myeloma globulin

Case EN

Electrophoretic and antigenic characteristics of serum and M-component

Agar gel electrophoresis of serum EN A demonstrated the presence of an M component of γ_2 mobility (Fig 1 d) Very little background gamma globulin seemed to be present

Paper electrophoretic quantitation of M component and background gamma globulin in serum EN A gave as result 3 8 and 0 07 g/100 ml, respectively

Starch gel electrophoresis of serum EN A together with purified M-component demonstrated for both preparations a pattern of at least 3 protein bands Comparison between the patterns showed the same characteristic spacing and position in the gel of the bands

Immunoelectrophoresis of serum EN A classified the M component as a GK protein

Gm groups in serum and M component EN The types found were for serum Gm (−1, −2, 4, 5) and for purified M component Gm (−1, −2, 4, −5)

Characterisation of the serum AST activity

Serum AST titre Repeated titrations on aliquots of serum EN A gave titre values between 49,000 and 105,000 The lastmentioned figure was obtained in an experiment where a duplicate sample gave the titre of 65,000 The discrepancy was thought to be due to a prozonal phenomenon (cf Chapter V) Serum EN B, gave the titre figure of 113 000 U/ml Serum EN C, titrated in duplicate showed a titre of 104,000—100,000, mean 102,000 U/ml

In the last experiment serum EN C was also titrated after heating at 56°C for 30 min giving titres in duplicate determinations of 34 000 and 37,000, mean 35,500 AST U/ml Hence, heat treatment reduced the serum AST activity to about 1/3 of that of untreated serum

Serum EN A was dialysed for 30 hr in the cold against 0 0175 M phosphate, pH 6 5 while another aliquot of the same serum sample stood at the same temperature for the same time An insignificant precipitate formed during dialysis The change in total protein concentration of the dialysed serum as obtained by comparison with the protein content of the control sample was therefore taken as a measure of volume change at dialysis When corrected for the volume change the titre of the dialysed serum nearly coincided with that of the control sample, *viz* 60,500 and 60,600, respectively (means of duplicate determinations)

Inhibition of serum AST activity by antisera (Fig 5) The experiments were set up as has been described for similar investigations on serum AO The results were definite inhibition by anti K, and almost complete inhibition by the 2 anti IgG sera No inhibition was noted with normal rabbit serum transferrin-anti-transferrin or anti L The controls without serum EN, *i e* the rabbit sera, transferrin and saline gave no definite inhibition of haemolysis

Preparation, characterisation and specific AST activities of IgG from serum EN

A pool of serum A and B was dialysed against 0 0175 M, pH 6 50 phosphate Of the serum 15 ml was chromatographed on DEAE-Sephadex A-50 The column (2×20 cm) had been equilibrated with the same buffer which

was also used for elution (flow rate approx 35 ml/hr) Protein emerged in one peak Most of the eluate was applied on a 2 × 20 cm column of CM Sephadex C-50 Fine , equilibrated with the same buffer This, *i e* 0 0175 M pH 6 50 phosphate, was initially used for elution The procedure was continued in 2 steps with 0 0175 M phosphate —0 03 M sodium chloride, pH 6 46 and 0 0175 M phosphate —0 04 M sodium phosphate pH 6 42, respectively Finally, gradient elution was carried through from the last mentioned buffer to 0 5 M sodium chloride as limit buffer The flow rate was approx 30 ml/hr

Little protein was obtained in the eluates at the stepwise and in the beginning of the gradient elution The pools of fractions from this part of the chromatogram are denoted IgG EN I—III Following the protein poor fractions a sharp peak, containing most of the eluted material came off the column The pool of fractions from the ascending part of the peak was designated as IgG EN IV, that from the middle of the peak as IgG EN V and the pool from the descending part as IgG EN VI IgG I contained very little protein, and was discarded whereas IgG II—VI were further analysed after concentration by ultrafiltration

Agar gel electrophoresis of the samples IgG II—IgG VI Aliquots of the samples were dialysed against distilled water and lyophilised Protein was then dissolved in 0 15 M sodium chloride to a calculated protein concentration of 0 35 g/100 ml and submitted to agar gel electrophoresis Serum EN A, diluted 1 4 with saline was used as a reference As seen from Fig 7 IgG EN V contains protein migrating

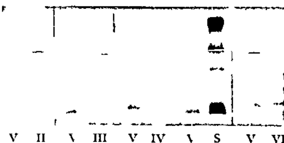


Fig 7 Agar gel electrophoresis of the chromatographic fractions IgG EN II—VI from serum EN and of diluted (1 4) serum Roman numerals indicate the fractions and S the serum The anode is at the top of the Figure

as the serum M component IgG EN II III and IV are seen to contain electrophoretically diffuse protein with a mean mobility higher than that of the M component On the original gels a faint protein stain extending into the M component region was obvious for IgG III and IV IgG VI definitely contained M-component and in addition some protein with a higher cathodal mobility

Immunochemical analysis of the fractions IgG EN II—VI was carried out as for IgG AO and IgG IN *i e* immunodiffusion against anti K and anti L sera and single radial immunodiffusion against anti IgG A and absorbed anti IgG EN sera, respectively IgG II, III and V were analysed in immunodiffusion against anti IgA and anti Ig M sera

AST titration was performed on the preparations IgG EN II—VI

IgG EN II III and V analysed against anti IgA and anti IgM sera at a protein concentration of 0 04 0 09 and 0 6 g/100 ml

Table 3 *Immunochemical and serological characteristics of IgG from serum EN*

Material IgG EN	II	III	IV	V	VI
K/L	2 7	2 0	4 0	>32	8 0
IgG % w/w	71	110	104	145	130
M-component % w/w	<13	31	52	100	67
Specific AST activity U/OD 80/ml	<5 8	<5 6	25	680	32

gave no precipitates, which, however, was the case with normal human serum diluted 1 10

Table 3 summarises the findings at immuno- and single radial immunodiffusion as well as the specific AST activities of the IgG EN preparations In the single radial immunodiffusion experiments with anti-IgG A serum presence of M-component, as in IgG EN V, evidently lead to overestimation of IgG The values of M-component in IgG EN II, III IV and VI found with anti IgG EN serum were probably unduly high. It was shown with the same antiserum that presence of polyclonal IgG (Kabi) expanded the ring from IgG EN V in mixtures of the 2 kinds of protein preparations IgG Kabi alone gave no visible reaction As seen from the Table there was co-variation of K/I ratios estimates of M-component and overshoot of IgG percentage on one hand, and specific AST activities on the other For IgG EN V the calculated contamination with background IgG was <0 0184/0 147 = 12 5 per cent; In the calculation the final concentrations of IgG EN III and V against anti-L have been used In conclusion, there was no sign of background IgG, IgA or IgM contaminating the M-component in IgG V The specific AST activity of IgG EN V, 680 AST U/OD_{280}/ml was therefore taken as the value of the specific AST activity that could formally be assigned to the M-component in IgG EN V

The specific AST activity of the M-component in serum EN A, taking the concentration of the M-component (3 8 g/100 ml) from the paper electrophoretic data together with the serum titre (105 000) could be calculated as 2000 AST U/OD_{280}/ml

The amount of M-component in IgG EN II was minor, judged from the result of the single radial immunodiffusion experiment with anti-IgG EN serum Hence the percentage of IgG in the preparation, as found with anti IgG A serum, was considered essentially correct Therefore the specific AST activity of background IgG in the preparation was calculated as <5 8/(71 per cent) = 8 2 AST U/OD_{280}/ml. Similar calculations for IgG EN III resulted in the estimate <8 6 AST U/OD_{280}/ml for the specific activity of background IgG

DISCUSSION

The decrease in haemolysis inhibition zone diameters, which was noted with some of the antisera employed in the blood agar lysin plate experiments with the patient sera AO, EN and IN is explained by one or both of 2 mechanisms Either the diffusibility or the anti-lysin activity of the serologically active principle of the patient sera is impaired In the control experiments immunoprecipitation *per se* or normal rabbit serum displayed a minor effect, or none at all Hence, the markedly inhibitive effect of the anti-IgG anti-K or the anti-I sera is probably specific In its turn, this probably implies that the AST or ASTA active principle in the patient sera is bound by the respective rabbit anti bodies Such binding should affect the diffusibility of the antilysin principle and should therefore lead to a decrease of the haemolysis inhibition zone diameter irrespective of whether the rabbit antibody impairs the anti lytic property or not Hence, the almost complete suppression of the haemolysis inhibition zone diameters from the sera AO, EN and IN with the anti IgG sera suggests that all, or lamost all of the antibody active principle in the patient sera consists of IgG The inhibition noted with the antisera against the light chain antigenic type of the respective M-components and the lack of effect with the antiserum against the other light chain type suggests that the serologically active IgG is of the M-component s light chain type Earlier findings (Zettervall et al 1966) and the present results from the preparative electrophoresis experiments substantiated that IgG accounts for practically all of the serological activity of the patient sera In these experiments the serological activity was almost quantitatively recovered from the fractions in the electrophoretic gamma globulin region which represents the bulk of the serum IgG The electrophoretic distribution of the antibody activity

inside the gamma globulin region strongly suggests that the M-components are the carriers of the activity. This receives ample support from the present findings with IgG preparations from the sera. Thus there is co-variation between indicators of M-component in the preparations on one hand and the specific antibody activity of the samples on the other. The higher the deviation in K/L or L/K ratio from that of polyclonal IgG or the higher the concentration of M-component as found with the anti individual IgG rabbit sera, the larger the specific AST or ASTA activities. Also in the case of EN and IN the overestimation of IgG with the anti IgG A serum, an effect which was seen to prevail in the M-components, correlates with the specific serologic activities.

In all 3 cases background IgG samples of comparatively low specific antibody activity have been obtained. This specific activity amounts to less than 1.5 per cent of that of the M-component in the case of EN, less than 1 and less than 10 per cent in the cases of AO and IN, respectively. For polyclonal AST it has been shown (Hedberg and Moritz 1960) that IgG from various chromatographic fractions of individual human sera displays a constant specific AST activity irrespective of the electrophoretic mobility of the protein. It is thus probable for the IgG preparations from the sera AO and EN that the specific activities found for the background IgG are representative for all the polyclonal IgG eluted from the columns. Thus the M-components AO and EN seem to account for the major part of the serological activity in the chromatographic fractions. In fact it could be calculated from the specific activity values found that more than 98.5 per cent of the AST activity in the prepared IgG should belong to the M-components. If the findings for the background IgG specific activity in the case of IN are regarded as typical, the M-component is calculated to account for more than 90 per cent of the ASTA activity in the preparations. Hedberg and Moritz also found coincidence between the calculated specific AST activity of IgG in the sera and that actually observed in their IgG preparations. These findings may suggest that the low specific activity figures found for the background IgG preparations of AO and EN also reflect a negligible specific AST activity of the native background IgG in the sera. Since the value of the specific AST activity of background IgG from serum AO (< 1.4 U/OD₂₈₀ ml) amounts to less than 1/5 of that of the pooled normal IgG (6.5 U/OD₂₈₀ ml) the background IgG may be of lower specific activity than is normally seen in polyclonal AST active IgG. Whether this also may apply to other antibody specificities in the 2 kinds of polyclonal IgG preparations is at present unknown. Presence of M-component in IgG EN III, IV and VI was demonstrated with the anti IgG EN serum. Calculating from these values and the calculated specific AST activity of the M-component in IgG EN V, the specific AST activities of IgG EN III, IV and VI become much higher than those actually observed. The same type of argument holds for IgG IN III. It may be that overestimation of M-component by the single radial immunodiffusion method is excessive or that M-component material in the fractions IgG EN III, IV and VI and in IgG IN III is of lower specific activity than that of IgG EN V and IN V, respectively. Earlier experiments (Zettervall 1967 b) indicating AST activity in several of the subfractions of the heterogeneous M-components failed to evaluate the differences in specific AST activity, if any, between the various components seen at starch gel electrophoresis.

The specific activities of the M-components from fractions IgG AO II, IgG EN V and IgG IN V lie far below the values calculated from titres and concentrations of M-component in the corresponding sera. The ratios between the figure from serum and that for purified material is about for AO and EN and 2 for IN. In the last case the ratio is even higher if the calculations are based on the titres of serum IN less aged than that used as source of IgG IN V. It is not likely that an

error in the paper electrophoretic quantitation technique is the explanation especially since the figure for the concentration of the M-component obtained from the preparative electrophoresis of serum IN (2 5 g/100 ml) nearly coincides with that of the other method (2 6 g/100 ml) As recovery of serological activity from the Sephadex block was nearly complete, the same should apply for protein in general, *i e* for the M-component Several other possible explanations are at hand, *e g* (i) damage of M-component at preparation (denaturation), (ii) loss of activity present in native background IgG, (iii) selective loss of AST active material, whether mono- or polyclonal, during preparation, or (iv) error in titre values due to a prozonal phenomenon

Hypothesis (i) is difficult to evaluate At least from gel filtration data it seems unlikely, that aggregation could play a major role (cf Chapter IV) (ii) This explanation is improbable in the light of Hedberg and Moritz' finding of equality between specific activities of purified polyclonal AST active IgG and that calculated for the serum gamma globulin (iii) A mechanism resulting in selective loss of antibody active material is hard to envisage for polyclonal AST active IgG The chances may be greater for M-component material, since its heterogeneity is less than that of background IgG, the selective loss of one very active subfraction may severely affect the specific activity of the preparation The differences seen in the relative intensities of the M-component sub-bands when serum and purified material are compared at starch gel electrophoresis, may be of importance in this context (iv) This topic is discussed in Chapter V

The results at agar gel electrophoresis of sera AO and EN show that AST active M-components of widely different electrophoretic mobilities occur (the M-component EN *has earlier (Zettervall et al 1966) erroneously* been described as of γ_1 instead as of γ_2 mobility) The M components AO and EN are of different light chain antigenic types and the same applies to the G myeloma globulins of the 2 sera with excessive AST activity recently described by Kronvall (1967) Thus the situation existing for IgM M-components in cases of the chronic cold agglutinin syndrome is not prevailing for the IgG M components with AST activity As is known such cold agglutinin active M components belong exclusively, or almost exclusively to one light chain antigenic type, *i e* K (Franklin et al 1963, Harboe and Deverill 1964)

There is a known strict relationship between Gm and heavy chain subgroup antigens (Kunkel, *et al* 1964) in that each Gm antigen is strictly limited to only one subgroup In the case of the M components discussed in this work all 3 are found from their Gm types to belong to the subgroup IgG 1 *A priori*, this is not an unexpected finding, since about 70 per cent of G-myeloma globulins belong to this subgroup (Grey and Kunkel 1964, Terry and Fahey 1964)

SUMMARY

The M component from the very high titred ASTA active serum IN and the highly AST active sera AO and EN is shown to contain antibody activity Antibody activity was not demonstrated in preparations of background IgG from the sera with the techniques employed If present the corresponding specific antibody activity is of the order of 1 per cent of that of the M component for cases AO and EN, at most 10 per cent for the case IN The specific serological activity of the purified M components is considerably lower, than that calculated from titre and concentration of M component in the sera The AST activity of sera AO and EN is heat labile whereas the ASTA activity of serum IN is not The serum IN activity is more sensitive to ageing than in the case of AO and EN The AST activity of serum AO is *sensitive to exposure to low ionic strength* phosphate buffer The Gm types of the 2 AST active M-components differ as do their antigenic light chain types All 3 M-components belong to the same heavy chain subgroup, IgG 1

III Properties of the papain fragments of IgG AO, EN and IN

The work of Porter (1959) on rabbit gamma globulin showed that papain cleaves the IgG molecule into 3 fragments of approx the same size Each of the 2 identical Fab fragments contains a heavy chain fragment (Fd) and most if not all of a light chain from the parent IgG molecule (Fleischman et al 1963) The third fragment i e the Fc fragment consists of the remaining halves of the heavy chain not present in the Fab fragment The Fc fragment is devoid of light chain material If the gamma globulin contains antibody activity only its Fab fragment retains the power to combine with the antigen after papain splitting of the IgG molecule The Fc fragment shows no antibody property (Porter op cit) Papain splits IgG from other species, among them man in principally the same way (Hsiao & Putnam 1961, Fougereau and Edelman 1965) Antibody activity has also been demonstrated in the Fab fragment of human IgG (Tan and Epstein 1963)

AST activity has been associated with the Fab fragment of IgG from sera with extremely high AST titres and IgG M-components (Kronvall 1967, Zettervall 1967 a) among them from the cases AO and EN The same pertains to Fab from IgG IN (last ref) The Fc fragments from IgG AO, EN and IN seemed devoid of antibody activity

Since the investigations on the papain fragments of IgG AO EN and IN were only qualitative an extended study of their properties seemed warranted This Chapter provides quantitative data on preparations of papain fragments from the 3 M components

MATERIALS AND METHODS

Preparation of IgG AO The preparation IgG AO II was described in Chapter II

EN, 5 ml of serum EN A, dialysed against 0 0175 M pH 6 50 phosphate was chromatographed on a 1 × 20 cm column of DEAE-Sephadex A-50 equilibrated and eluted with the same buffer Flow rate was approx 5 ml/hr

IN, 5 ml of serum IN was separated with the same technique When analysed at a protein concentration of 0 75 g/100 ml by agar gel electrophoresis the proteins appeared as pure M-components

Preparation of light chains The same preparation from normal human IgG as described in Chapter IV was used

Determination of protein concentration Chromatographic fractions were assayed for protein by spectrophotometry at 280 nm All other protein determinations were carried out with the Lowry method

Agar gel electrophoresis Protein samples in 0 0175 M pH 6 50 phosphate buffer were mixed with 1/20th volume of 2 M tris-HCl buffer pH 8 0 before electrophoresis The addition was made in order to prevent distorsion of the electrophoretic pattern which otherwise was apparent

Antisera The following antisera already described in Chapter I were used in immunoelectrophoresis and in inhibition experiments antiserum against normal human IgG absorbed with normal human light chains (*anti IgG A*) antiserum against normal human heavy chains absorbed with light chains (*anti γ chain*) antiserum against normal human light chains, absorbed with heavy chains (*anti-light chain*) antisera against IgG from sera AO EN and IN (*anti-IgG AO, anti IgG EN and anti-IgG IN*) All sera used in the antibody inhibition experiments were dextran precipitated

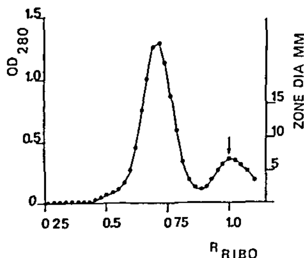


Fig 1 Gel filtration in Sephadex G 200-phosphate buffered saline of papain digest of IgG AO and subsequent chromatography in the same column of fraction AO D from preparative electrophoresis First experiment OD280 (ordinate left full line) of fractions Second experiment. Haemolysis zone inhibition diameters in mm (ordinate right dots) at titration of the fractions in streptolysin blood-agar diffusion plate The abscissa gives the elution volumes relative to that of riboflavin Arrow denotes the riboflavin peak

Immunoelectrophoresis was mostly performed as described in Chapter I Sometimes a comparative method (Osserman 1960, Wadsworth and Hanson 1960) was used

Papain digestion of IgG Solutions of IgG in 0 0175 M, pH 6 50 phosphate buffer and containing 15 mg protein per ml were mixed with 1/10 volume of 0 02 M ethylenediaminotetraacetic acid, disodium salt (EDTA-Na_2) and 1/10 volume of 0 01 M cysteine, both in the mentioned phosphate buffer Mercuri papain (Worthington Biochemical Corp, Freehold, New Yersey USA batch No 6113) contained in less than 1 20 volume of ethanol was then added The protein to enzyme ratio was 1 100 (w/w) Digestion was carried out at 37 C for periods as described below Digestion was stopped by addition of 1 original protein sample volume of 0 001 M para-chloromercuribenzoate in phosphate buffer Control samples of IgG EDTA Na_2 and cystein in the same proportions as used in the digestion mixtures but without papain were prepared They were incubated and then mixed with para chloromercuribenzoate as was the case with the mixtures containing papain

RESULTS

Action of papain on the M components Papain digestion of the M-components was carried out for various time periods The digests were analysed by agar gel electrophoresis together with control IgG samples If papain was allowed to act for a sufficient time the digests were seen to contain a fast and a slow electrophoretic component indicating splitting of the IgG The susceptibility to papain action was markedly different for the 3 M components IgG AO was very rapidly degraded Thus after 10 minutes digestion the fast and slow components were present but undigested IgG could not be detected Digestion for longer periods reduced the intensity of both electrophoretic components and the fast fragment was barely visible after a half hours papain treatment The protein IN was more resistant and little cleavage seemed to have occurred after 10 min After 30 min however, only traces of undigested IgG was discernible whence strong fast and slow components were apparent Further digestion slowly reduced the intensity of both components IgG EN was the least papain sensitive Fast and slow components were barely seen after 10 min digestion After 1 hr much of the M-component remained but was virtually absent after 4 hrs At this time much of the fragments were still present

Preparation of papain fragments IgG AO, IgG EN and IgG IN were digested for 10, 30 and 240 min, respectively The samples, originally containing 30—45 mg IgG, were concentrated to approx. 1 2 ml mixed with riboflavin and gel filtered in Sephadex G 200 equilibrated with PBS under the conditions described in Chapter I Column dimensions were 0 9—1 1 × 90 cm The result is illustrated in Figure 1 In all 3 cases a major protein peak with its

maximum at R_{ribo} 0.715—0.740 was evident The peaks were preceeded by insignificant shoulders in the region of IgG (R_{ribo} 0.550—0.575), as determined by gel filtration of radioiodinated M components (Chapter IV) Pools of fractions were collected from the central parts of the major peaks

The pools were concentrated to 0.5—1 ml by ultrafiltration Most of the material was then fractionated by preparative electrophoresis in Sephadex G-25 blocks

Electrophoresis was carried out for 15—36 hrs varying in the different cases A representative result is given in Figure 2 From all preparations a protein rich and slowly migrating protein fraction was obtained and in addition a faster and complex peak of lower protein concentration From each protein peak 2 pools of fractions were taken as illustrated by the Figure The pooled materials were analysed as described below

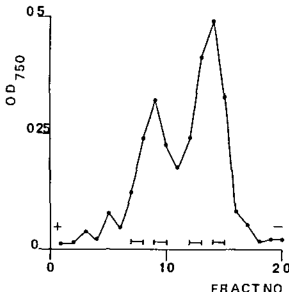


Fig 2 Preparative electrophoresis in Sephadex G 25 block of gel filtered papain digest of IgG AO The ordinate gives the optical density at 750 nm obtained at protein determination according to Lowry The markings above the abscissa denote pooling of fractions (preparation A extreme left—D extreme right)

Serological, immuno and physicochemical characterisation of the electrophoretic fractions

Approximately 2/3 of each pool of fractions from the preparative electrophoresis was concentrated approx 10 fold by ultrafiltration This material control IgG preparations and aliquots of the unfractionated digests were analysed by immuno electrophoresis The protein concentrations of the fractionated and concentrated material varied between 0.026 and 0.38 g/100 ml The protein concentration of the fractions C and D were 2—13 times higher than that of the respective fractions A and B When the digests the pools A—D and the control IgG preparations were analysed with the anti IgG AO EN and IN sera the digests were seen to contain fast and slow components with electrophoretic mobility higher and lower than that of undigested IgG respectively In the case of the digest of IgG AO absence of cross reaction between the fast and slow fragment was evident (Figure 3 a) Cross reaction or its absence was difficult to delineate for the components of the other digests since there was material present between the components with the mobility of undigested IgG At least the fast components cross reacted with the IgG like material When the pools A—D were examined with the same antisera (shown for AO in Figure 3 b and c) slow component was demonstrated in the pools C and D but not in A and B In addition the fractions C and D were in each case seen to contain material precipitating near the antigen well This material will provisionally be referred to as the well near component In the fraction C of AO the well near component predominated and only a faint precipitin line from the slow component was discernible The pools A and B from AO and EN gave wavy precipitin lines, indicating presence of 2 subfractions In mixtures of A and D materials absence of cross reaction between the fast and slow components was noted The same applies to the fast and "well near' components in the case of AO In this cross reaction between the slow and 'well near' component was obvious The

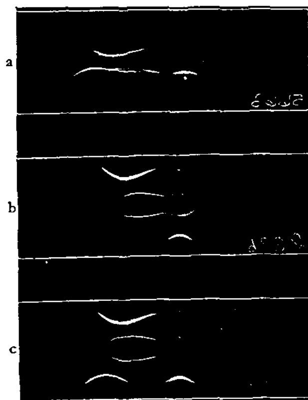


Fig 3 Immunoelectrophoresis of control IgG AO preparation papain digest of IgG AO and preparative electrophoresis fractions A—D from the gel filtered digest Anti IgG AO serum in the troughs Slide a) control IgG (top) digest (below) Slide b) control IgG (above) fraction A (centre) fraction C (below) Slide c) control IgG (above) fraction B (centre) and fraction D (below) The lower precipitin line seen in slide b) represents the 'well near component it occurs in a similar position in slide c)

'well near' component in the EN and IN material was too faint to allow conclusions regarding cross reactivity Reaction against undigested IgG was discernible even at a protein concentration amounting to 1/10 of that of AO D 1/40 of that of EN D and 1/35 of that of IN D The anti-IgG A and the anti γ-chain sera precipitated the fast but not the slow or the well near components The anti light chain serum gave precipitin lines against the slow components from EN and IN but not with certainty against the slow material in AO D However, employing anti light chain serum and with light chains in the slit opposite the antiserum basin, the slow AO component was seen to react Reaction was not obvious between the anti light chain serum and the "well near" components The anti light chain serum gave no reaction against the fast components with either of the techniques

As described below the materials C and D were seen to contain antibody activity which was absent or weak in the other fractions

In order to elucidate the nature of the active principle in the pools D, these were investigated in inhibition experiments with the antisera using the plate titration techniques The antisera employed were anti IgG A and anti-light chain Normal rabbit serum was used as a control and in addition transferrin plus anti-transferrin for AO and EN material and BSA plus anti BSA for IN material, as unspecific precipitate controls Other controls were serial dilutions (saline) of the material to be tested, transferrin, BSA, the rabbit sera and saline All preparations applied to the plates contained dextran sulphate and calcium chloride at the same concentrations Equal amounts of D material and rabbit normal or antiserum or saline were mixed before application onto the plates For AST determination 5 μl applications were used and 10 μl applications for ASTA assay The D material was present in the mixtures at a protein concentration of 0 002 g/100 ml and the same concentration was chosen for transferrin and BSA The results were in all cases the anti-IgG A serum

gave a moderate decrease in haemolysis inhibition zone diametres whereas the anti-light chain serum inhibited almost completely The unspecific precipitation controls were negative with the exception of AO material where some decrease in zone diameter was obvious The decrease was however, less than that given by the 2 other sera Normal rabbit serum gave no depression in haemolysis inhibition The other control preparations did not inhibit haemolysis

The D materials were then gel filtered and the chromatographic fractions assayed for antibody activity with the plate methods (shown for AO in Figure 1) No antibody activity was demonstrated at the characteristic position of IgG in the chromatogram Instead all antibody activity was localised in a peak, the R_{ribo} of which exactly coincided with that of the main protein peak from the whole digests previously analysed in the same respective columns At tube titration no antibody activity was observed in the region of IgG whereas activity in the peak region was confirmed

Table 1 gives the result of titrations on the unconcentrated materials A—D together with determinations on the control IgG preparations In each case the specific AST or ASTA activity of the C and D materials exceeded that of the A and B fractions The first mentioned materials gave approximately the same value These were for AO and IN approx 80—100 per cent of the value for the control IgG, whereas the ratio was approx 5 per cent for the EN fractions In no case was activity demonstrable in the A fractions, in spite of subsequent concentration

In another experiment the fractions A, or A plus B D and mixtures of the fractions were titrated (Table 2) The A or A plus B fractions were present in 4- to 8-fold excess (w/w) over the D fractions in the mixtures No activity was proved in the A or in the A plus B fractions and these materials in the mixtures did not inhibit the activity of the D fractions

Table 1 *Specific AST or ASTA activities (U/OD280/ml) of fractions from preparative electrophoresis and of control IgG preparations*

Material	Case AO	EN	IN
IgG	1060	811	14
A	<8 7	<3 1	<0 83
B	48	<8 0	<0 53
C	1000	33	12
D	770	42	16

No antibody activity was discernible in the fractions AO EN and IN A or in EN and IN B

DISCUSSION

Gel filtration of the papain digests showed that almost all IgG had been cleaved by the enzyme The rapid degradation of IgG AO is remarkable and contrasts to the relative stability towards enzymatic attack shown by the other AST active M component The susceptibility of IgG IN to papain action was intermediate compared to that of IgG AO and EN The slow fragment, demonstrated in the preparative electrophoresis fractions C and D and virtually absent from the fractions A and B was shown to consist of the Fab fragment as judged from its electrophoretic mobility the absence of precipitin reaction with the anti IgG A and the anti-γ chain antisera (Cohen 1963) and its demonstrated content of light chains The fast fragment, prevailing in the electrophoretic fractions A

Table 2 *AST or ASTA titre of fractions and mixtures of fractions from preparative electrophoresis*

Case	AO			EN			IN		
Material	A	D	A+D	A+B	D	A+B+D	A	D	A+D
Titre U/ml	<1	1 5	1 9	<1 3	1 2	1 2	<1 7	4 5	4 5

and B, was diagnosed as a Fc fragment by its electrophoretic mobility and its precipitin reaction with the anti-IgG A and the anti-γ-chain sera As expected, cross reaction between the fast and slow components was not demonstrated The "well near" component was shown in the case of AO to cross react with the Fab fragment, and absence of cross reaction with the Fc fragment was obvious The nature of the fragment is unclear, but it may possibly be an Fd fragment

AST or ASTA activity was demonstrated in the C and D fraction, and the B fraction from AO but in neither case in the A fraction The antibody activity in the D preparations could not be due to undigested IgG This was shown by the gel filtration experiments, where activity was only demonstrated in material of lower molecular size than that of IgG The immunoelectrophoretic findings were also incompatible with the presence of IgG in amounts sufficient to account for the observed antibody activities in the D preparations The activity in the fraction AO C, which seemed to contain little Fab fragments suggested in this case that the 'well near" component contained AST activity The marked suppression of haemolysis inhibition zone diameters obtained with the anti light chain antiserum in all cases suggests that the Fab fragments were antibody active

The A preparations, by immunoelectrophoretic criteria consisting of pure Fc fragments, showed no AST or ASTA activity This also applies to the fraction EN B Even when present in molar excess the Fc fragments did not block the antibody activity from the D preparations

The specific activity of the EN C and D materials was lower than that of the control IgG preparation This means that all of the AST activity of the IgG molecule was not accounted for by the C and D material The discrepancy may be misleading, however, since the AST activity only represents secondary manifestations of the reaction between lysin and the M component, *i e* inhibition of lytic enzyme activity Thus a comparatively low recovery of AST activity may be compatible with a quantitative recovery of active sites This explanation is unlikely in the case of the Fc fragments, since, as mentioned, they were unable to block the action of the D material The finding of antibody activity in the Fd half of the M components implicates that these IgG molecules are at least bivalent

SUMMARY

Preparations of Fc and Fab fragments were obtained by gel filtration and preparative electrophoresis of papain digests of IgG AO EN and IN The Fc fragments seemed pure while the Fab fragments were contaminated with unidentified material In the case of AO this material was shown to be antigenically related to the Fab fragments but unrelated to the Fc fragments With the techniques used the Fc fragments proved antibody inactive, whereas activity was demonstrated in the Fab fragments In the case of AO the unidentified material was probably antibody active

IgG AO was highly susceptible to papain action, IgG EN was relatively resistant and IgG IN of intermediate susceptibility

IV Properties of the polypeptide chains of the M-components AO, EN and IN

As has been pointed out in the Introduction studies have earlier (Sjoquist and Zettervall 1966, Zettervall 1967 b) been carried out on the properties of the polypeptide chains of the three M components Thus the specific antibody activity of the heavy and light chain preparations was found to amount to approx one per cent of that of the respective M-component preparations On comparison the specific AST activity of the heavy chains of the M-component EN was found to probably exceed that of the light chains from the same source On the contrary several experiments with the material AO seemed to indicate a slight excess of specific AST activity in the light chains over that of the heavy chains No definite conclusion was reached as regards the ratio of specific activities of heavy and light chains from the M component IN because of the low levels of antibody activity encountered in analysis of this material The possibility could however not be ruled out that the antibody activity of the polypeptide chains was due to contamination with complementary chains or undissociated IgG In recombination experiments with autologous heavy and light chains from the three M-components, potentiation of antibody activity was noted in each case When heavy and light chains from the same IgG preparation were mixed and the dissociating condition (acid pH) removed, the specific antibody activity of the mixture was found to markedly exceed the specific activity value, calculated from that found in isolated preparations of the chains and the known concentration of these in the reconstituted mixture Potentiation of antibody activity in conditions known to favour recombination of chains to four chain molecules is a typical finding for animal antibodies (Franěk and Nezlin 1963, Edelman et al 1963)

In homologous recombination experiments, where chains from AO and EN were used together with complementary chains from other IgG, comparatively little, or no potentiation was encountered No definite advantage was obtained in crosses where both complementary chains were derived from AST active IgG, either poly- or monoclonal The polyclonal chains used in these investigations (from IgG prepared from serum SE) gave minor or no potentiation in autologous recombination experiments Hence, in recombination experiments the M-component material seems to yield to the pattern considered as characteristic for antibodies, whereas the polyclonal human preparation SE does not However the relatively high specific antibody activity of the light chain preparation AO compared to that of the autologous heavy chains may disturb the analogy with the animal antibodies For these, heavy chains have usually been found to possess higher antibody activity than the complementary chains (Fleischman et al 1963, Utsumi and Karush 1964, Porter and Weir 1966) although exceptions from this rule seem to exist (Goodman and Donch 1965)

So far, the antibody active material in reconstituted chain mixtures from the M-components AO, EN and IN have not been characterised in physico- and immunochemical terms This was the object of the investigations described in the present Chapter *i e* identification of the antibody active principle formed in such recombination experiments Similar investigations are also given on the antibody active material in iso-

lated chain preparations from the M components under study

MATERIALS AND METHODS

IgG from serum EN A was prepared by chromatography in DEAE-Sephadex A-50 with phosphate 0 0175 M, pH 6 50 as starting and eluting buffer IgG GW was prepared by the same technique from a human G myeloma serum The preparation had a specific AST activity of <1 AST U/OD_{280}/ml IgG from serum AO B was obtained by chromatography in DEAE-Cellulose (Serva) using the same buffer, 5—15 ml of the sera were fractionated Column dimensions were 2×20 cm and flow rates 10—30 ml/hr IgG from serum IN was the preparation IgG IN V, described in Chapter II When the samples were analysed at a protein concentration of 1 g/100 ml in immunoelectrophoresis with polyvalent anti-human serum, only IgG could be demon strated During cellulose acetate electrophoresis employing the same protein con centration the preparations seemed to contain pure M-components IgG Kabi was used as a source of normal polyclonal heavy and light chains

Radio iodine labelling of IgG was carried out according to Bocci s (1964) modification of Hunter and Greenwood's (1962) Chloramine T method The Chloramine to protein ratio was 1 200 and reaction time 30 min After addition of carrier iodide, free radioisotope was removed by gel filtration in Sephadex G-25 "Fine (column 1×17 cm 0 0175 M, pH 6 50 phosphate, flow rate appr 15 ml/hr) The average extent of labelling was less than 1 iodine atom per molecule of IgG The specific radioactivity of the labelled IgG preparations was between 10^5 and 10^7 counts per minute/unit absorbency at 280 nm (cpm/OD $_{80}$/ml) With the exception of IgG Kabi and IgG GW which were not iodinated, one aliqu of each IgG preparation was labelled w ^{31}I and another at the same time with ^{125}I

Preparation of heavy and light chains Reduction (tris-HCl 2 M, pH 8 0, 2-mercaptoethanol 0 5 M, 1 hr) and alkylation (iodo acetic acid 0 55 M in same buffer 20 min) have been described elsewhere (Zettervall 1967 b) Part of the material was dialysed against 0 05 M tris 0 15 M NaCl, pH 7 2 The remainder was dialysed against 0 1 M formic acid in the cold for 18—30 hr after which heavy and light chains were separated by gel filtration in Sephadex G-200 40—120 μ in the same buffer (Sjoquist and Vaughan 1966) In the experiments with monoclonal IgG the column dimensions were 1 5×85 cm and the flow rates between 2 and 3 ml/hr Chains from IgG Kabi were separated in a 4×80 cm column at a flow rate of approximately 20 ml/hr Fractions were taken each hr and read at 280 nm and appropriate pools of fractions corresponding to the respective heavy and light chain peaks were collected For each IgG material ^{131}I and ^{125}I labelled chains were prepared simultaneously in parallel columns

Acrylamide gel electrophoresis was used for characterisation of heavy and light chain preparations Chain preparations in 0 1 M formic acid or mixtures of chain preparations of an OD_{280} of 0 4—0 7 were concentrated 5- to 10 fold by lyophilisation and subsequently dissolved in urea SDS phosphate sample buffer (Chapter I) Part of the preparation was saved for counting while other aliquots were electrophoresed under the conditions described in Chapter I When radioactive material had been thus fractionated, 0 2 cm gel segments were cut, using a metal comb as a spacer IgG preparations were lyophilised from 0 0175 M, pH 6 50 phosphate buffer and dissolved in ½—1 volumes of sample buffer before electrophoresis In one experiment, IgG EN in the same phosphate buffer was mixed with 10 volumes of 0 1 M formic acid and kept overnight at room temperature before lyophilisation and electrophoresis at its original protein concentration

Recombination of polypeptide chains Heavy (H) and light (L) chains in 0 1 M formic acid were mixed at the mass ratio H/L of 4/3 as deter-

mined from measurements of the OD_{280} of the chain preparations The same specific extinction coefficient *i e* 13 6, was assumed for the 2 kinds of material The optical densities ranged between 0 2 and 0 6 The mixtures, and preparations of the chains used in the mixtures, were simultaneously dialysed in the same bath for 36—50 hr at +5°C At least a hundredfold volume of 0 05 M tris 0 15 M sodium phosphate pH 7 2 was used as outer solution the dialysis buffer was changed once Protein concentration was determined by spectrophotometry at 280 nm, using the last dialysis buffer portion as a blank The AST or ASTA activity of the samples was determined and their specific antibody activity in AST or ASTA U/OD_{280}/ml was calculated In some instances, the dialysed chains were mixed and the preparations analysed simultaneously according to the same principles Potentiation of antibody activity in chain mixtures was calculated from the formula

(observed specific antibody activity of chain mixture)/(calculated specific activity of mixed chains)

The denominator is calculated from the sum of the antibody activities of the chains in the mixture, using the figures for the specific antibody activities of the isolated chain preparations and the known proportion of heavy and light chains in the mixture

Gel filtration Protein samples in neutral buffers, such as radio-iodinated IgG, reconstituted mixtures of heavy and light chains or isolated chain preparations in such buffers were analysed by gel filtration in Sephadex G 200 40—120 μ equilibrated with PBS The riboflavin marker (Chapter I) was always included in the samples to be fractionated Before application of the samples (0 5—1 0 ml) to the columns an aliquot of the material had been removed for counting 3 columns of diameter 0 9—1 1 length 90 cm were run as described in Chapter I In the autologous recombination experiments dialysed chain preparations and chain mixtures were chromatographed simultaneously The same applies to the 2 kinds of chain mixtures in the homologous experiments In these, however, free chain preparations were usually not gel filtered

The anti-light chain anti-IgG A and anti-IgG AO antisera have been described in Chapter I Anti IgG AO was absorbed with an excess of polyclonal normal human light chains The sera were not delipidised

Serological methods AST activity was usually titrated with the ordinary tube method or, in some instances, with the micro tube method The choice of method is specified in each instance ASTA activity was determined by the tube method The plate methods were used for identification of antibody activity in chromatographic samples and in inhibition experiments with antisera In the AST inhibition experiments equal volumes of antiserum and test solution were mixed 5 μl of the mixture was then immediately applied onto the blood agar diffusion plate

Conventions Chain preparations are referred to by self-explanatory abbreviations *eg* H(AO) L(EN), etc. If needed the symbols include the iodine label *e g* H_{125_I} Iodine labelled IgG preparations are similarly denoted Chain mixtures are given as $H_{131_I} + L_{125_I}$, etc. Preparations of chains mixed in formic acid and subsequently dialysed against tris NaCl buffer will be referred to as chain mixtures or reconstituted chain mixtures Preparations of chains mixed after previous dialysis are denoted as mixtures of chains

RESULTS

Preparation and analysis of heavy and light chains

Separation of heavy and light chains Fig 1 is representative for the elution curves and pooling of fractions in the heavy and light chain separations The variation noted in the shape of the elution curve in different experiments affects mainly the leading composite peak and probably reflects different degrees of heavy chain aggregation and/or presence of undissociated IgG In collecting heavy chains,

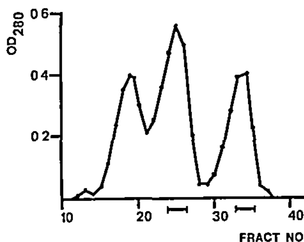
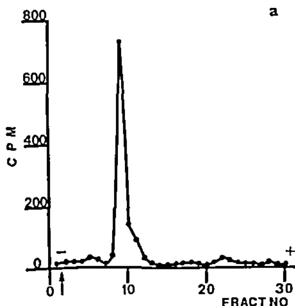
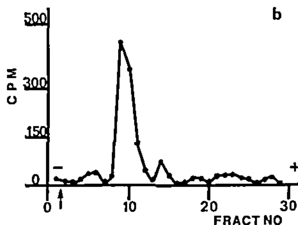


Fig 1 Separation of polypeptide chains from reduced and alkylated IgG EN (20 mg) in Sephadex G 200-0.1 M formic acid. Column dimensions were 1.5 × 85 cm and fraction volumes 2.6 ml

the ascending part of the leading peak was avoided

Acrylamide gel electrophoresis of IgG and of chain preparations Fig 2 a–c gives representative counting patterns obtained when iodine labelled IgG and chain preparations from the same protein were electrophoresed in the acrylamide-urea-SDS system. The samples, analysed in the same gel, were in this case L_{131I} (EN) mixed with approx 1/10 (w/w) H_{125I} (EN), ¹³¹I labelled IgG EN, and the





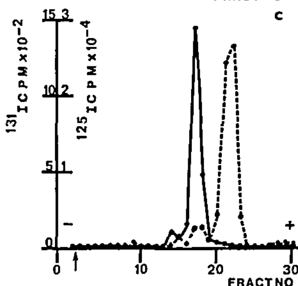


Fig 2 Acrylamide gel electrophoresis of a) IgG (EN)$_{131I}$ b) IgG (EN)$_{131I}$ treated with formic acid c) a mixture of H_{125I} (EN) and L_{131I} (EN). The radioactivity of the gel fractions is given as counts per minute (cpm) on the ordinate. ¹³¹I radioactivity is denoted by the full line in a) and b). In c) the full line denotes ¹²⁵I and the broken line ¹³¹I radioactivity

same type of material, treated with 0.1 M formic acid as described in the Materials and methods section of this Chapter. As seen from the Figure the most anodic principal radioactivity peak from the L chain preparation was well separated from the main heavy chain peak. IgG gave a peak of mobility lower than that for heavy and light chains. Formic acid treatment did not grossly affect the mobility of the protein. The heavy chain preparation in addition showed one minor peak with lower electrophoretic mobility than that of the main fraction and the same applies to both

IgG preparations The small peaks probably represent aggregated material The IgG preparations gave shallow peaks in the heavy and light chain positions The slow minor peak from L_{131_I} corresponded in mobility to heavy chains and was thus probably due to contamination with such material If present a light chain peak from the heavy chain material was insignificant No definite peaks from the chain preparations in the typical IgG position were discernible Similar results were obtained with the other chain preparations The chain preparations of IgG GW and pooled normal IgG were seen to contain insignificant amounts of contaminating complementary chains or IgG, as judged from the protein stain pattern (Fig 3) When H (GW) and L (GW) were analysed at the same time with iodine labelled chains from IgG AO, or with unlabelled polyclonal chains, the electrophoretic mobilities of the respective chains from the different preparations correlated closely However, on comparing the stain patterns the heavy and light chain stain from the normal IgG preparation seemed more diffuse than those from IgG (GW)

The total recovery of radioactivity in the gel fractions amounted to between 80 and 95 per cent of the activity applied to the plate Calculation of the contamination with complementary chains was facilitated by the addition of the samples with chain preparations, labelled with the alternative iodine isotope This is illustrated by the following example which shows the method of calculating the degree of contamination of L_{125_I} chains with $H_{1\,3_I}$ H_{131_I} is assumed to have been added to the preparation The specific radioactivity (cpm/unit mass) is first assumed to be equal for both H_{125_I} and L_{125_I} If the H_{131_I} peak contains p_{131_I} per cent of the total ^{131}I label applied to the plate and p_{125_I} per cent of the ohter label is found in the heavy chain position the mass ratio H_{125_I}/L_{125_I} in the sample is calculated as p_{125_I}/p_{131_I} In this calculation the chain preparations have been assumed as

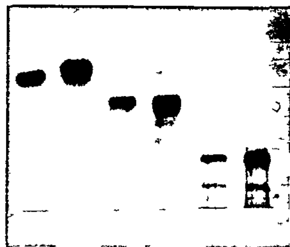


Fig 3 Electrophoresis in acrylamide gel of polypeptide chains from IgG GW and pooled normal IgG From left to right L (GW) normal L chains H (GW) normal H chains IgG (GW) and normal IgG The anode is at the top The protein content of the samples was 0 15 mg

nearly pure With the specific radioactivity of $H_{1\,3_I}$ and L_{125_I} denoted as s_H and s_L, respectively and now disregarding the restriction $s_H/s_L = 1$, the percentage of contaminating H_{125_I} chains in the L_{125_I} preparation is $(p_{1\,2_I}/p_{131_I}) \times (s_L/s_H) \times 100$ The contamination with IgG was calculated from the radioactivity recovered from the position of IgG This position as well as the percentage of recovery of the total IgG radioactivity in the gamma globulin peak was obtained at parallel analysis of IgG in the same plate

The contaminations of the chain preparations are given in Table 1

Characterisation of IgG and of dialysed chains and autologous chain mixtures

The $IgG_{1\,3_I}$ preparations were characterised by gel filtration.

IgG iodine labelled IgG and reduced, alkylated but not dissociated labelled IgG were titrated simultaneously and their specific antibody activities calculated from optical density readings at 280 nm. The IgG preparations AO and EN were titrated both with the ordinary and the micro tube techniques

Table 1 *Contamination of chain preparations with IgG and complementary chains*

Case	Chain preparation	Contaminating material (per cent w/w) H-chains	L-chains	IgG
AO	$H125_I$		14	0 7
	$L125_I$	1 1		0 04
	$H131_I$		13	0 9
	$L131_I$	1 5		0 07
EN	$H125_I$		2 3	0 2
	$L125_I$	0 9		0 05
	$H131_I$		2 8	1 1
	$L131_I$	12		1 3
IN	$H125_I$			0 1
	$L125_I$	10		0 02
	$H131_I$			0 8
	$L131_I$	1 3		2 2

Chain preparations and chain mixtures were analysed as described in Materials and methods In the case of AO and IN, mixtures of dialysed chains were similarly investigated, but only the IN material was gel filtered

Gel filtration of iodine labelled IgG AO (Fig 4) *EN and IN* The radioactivity peaks emerged at a R_{ribo} from 0 550 to 0 575

Presence of aggregated IgG was evident from the shape of the radioactivity curves at the leading part of the main peaks Aggregated radioactive material seemed to constitute only a minor part of the total iodine labelled protein recovered from the columns The recovery of radioactivity in the fractions was 60 to 100 per cent of that applied to the columns This contrasted with the recovery of heavy and light chain radioactivity in the subsequent experiments where the figures were much lower, i e less than 50 per cent

Case AO

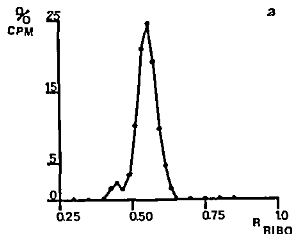
Specific AST activity of IgG and of reduced and alkylated iodinated IgG (Table 2 Experiment 1) Without iodination IgG had a specific AST activity by the ordinary tube method nearly coinciding with that of IgG AO II in Chapter II With the micro modification approx half the value was obtained Iodine labelling did not adversely affect the AST activity, on the contrary, compared with untreated IgG the figures for the iodinated proteins were slightly higher using the ordinary titration method However, this difference is not considered definite As for untreated IgG, the titres obtained with the micro method were lower than those found at ordinary titrations Reduction and alkylation decreased the specific AST activities to about 35 per cent of the figure for iodinated IgG, when the results found with the ordinary titration method are considered There was no real difference between the specific AST activities obtained

Table 2 *Case AO Specific AST activity (U/OD280/ml) of IgG polypeptide chains reconstituted chain mixtures and mixture of dialysed chains*

Exp	Material	Specific AST activity		Potentiation	% recovery of Ab activity
1	IgG	1760	*909*		
	$IgG125_I$	1865	*835*		
	$IgG125_I$ r+a	704	*833*		
	$IgG131_I$	2133	*1342*		
	$IgG131_I$ r+a	743	*624*		
2	$H125_I$		*4 52*		
	$L125_I$		*27 7*		
	$H131_I$		*11 8*		
	$L131_I$		*15 5*		
	$H125_I+L131_I$		*113*	12 3	18 1
	$H131_I+L125_I$		*297*	16 0	47 6
	* $H131_I+L125_I$		*20 2*	1 0	
3	$H125_I$		*76 4*		
	$L125_I$		*13 5*		
	* $H125_I+L125_I$		*32 1*	3 0	

Figures in italics denote results from micro tube titrations r + a = reduced and alkylated
* mixture of dialysed chains

with either titration method, in contrast to that found for untreated or iodine labelled IgG *Specific AST activity of heavy and light chains, chain mixtures and and mixtures of chains* (Table 2, Experiment 2 3) In Experiment 2 chains and reconstituted chain mixtures from the 2 iodinated IgG preparations were dialysed together The samples were titrated together with a mixture (H/I = 1/1, w/w) of aliquots from the dialysed chain preparations H_{131_I} and L_{125_I} The specific AST activity of H_{125_I} was much lower than that of H_{131_I} The reverse is true for the respective L chain preparations For both sets of complementary chains the specific AST activity of the light chains exceeded that of the heavy chains The specific activity of any chain preparation was less than 4 per cent of that of reduced and alkylated IgG For both reconstituted mixtures *i e* $H_{125_I} + L_{131_I}$ and $H_{131_I} + L_{125_I}$ the specific AST activity figure was several times higher than the values found for the isolated chain preparations which is illustrated by the potentiation factors, 12 3 and 16 0 In the combination $H_{131_I} + L_{125_I}$ this activity nearly amounts to 50 per cent assuming that all heavy chains in the mixture recombine with light chains to four chain molecules of specific activity equal to that of reduced alkylated and undissociated IgG In the case where the chains were mixed after



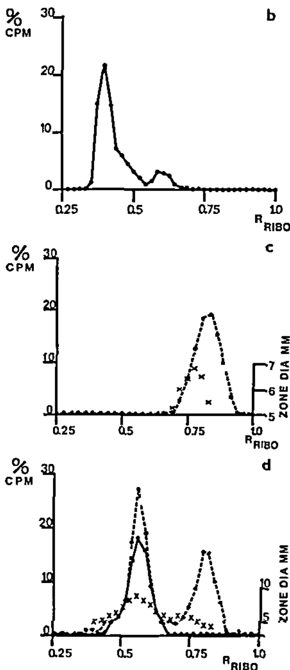


Fig 4 Gel filtration in Sephadex G 200-PBS of iodine labelled IgG AO dialysed polypeptide chains and a reconstituted chain mixture The abscissa gives the elution volumes relative to that of riboflavin Left ordinate radioactivity from ^{125}I or ^{131}I as percentage of the total radioactivity recovered from the column of the respective isotope right ordinate zone diameters at AST plate fibration a) IgG b) H_{131_I} chains c) L_{125_I} chains and d) reconstituted mixture $H_{131_I}+L_{125_I}$ In b) c) and d) full line denotes radioactivity from ^{131}I and broken line that from ^{125}I Crosses AST activity

previous dialysis no enhancement of AST activity was found as seen for the combination, $H_{131_I} + L_{125_I}$ However, in Experiment 3, where dialysed chains were mixed in the proportion (w/w) H/L = 0 88 potentiation was found

Characterisation of dialysed chains and chain mixture by gel filtration (Fig 4) The dialysed chain preparations H_{131_I}, L_{125_I} and the chain mixture, dialysed after mixing of the chains in formic acid, were analysed by gel filtration These preparations are the same as those given in Table 2, Experiment 2

The heavy chain preparation H_{131_I} gave a main radioactivity peak with a R_{ribo} of 0 395 followed by a minor peak with R_{ribo} of 0 595 Both peaks appear complex Plate titration showed a questionable activity in a few fractions preceeding the principal peak and a weak, but definite activity in the region R_{ribo} 0 375—0 585, with a maximum at 0 457 Titration with the micro tube method showed one activity peak at R_{ribo} = 0 30 Since the radioactivity of this fraction, *i e* the one which was immediately followed by the first definitely radioactive fraction of the main peak, was not significantly higher than the background, no specific AST activity could be calculated Hence the heavy chain preparation seemed to contain 2 AST active fractions one poorly diffusible but more antibody active than the other, more diffusible fraction

The light chain preparation L_{125_I}, gave one slightly skewed count peak with maximum at R_{ribo} = 0 834 AST activity was clearly demonstrable in the region 0 611—0 863 with a maximum at 0 777 This is near the characteristic position of the AST activity peak (0 715—0 740) given by Fab fragments (cf Chapter III) These findings were confirmed at tube titration A specific AST activity was calculated using the titre found for the most AST active fraction and the specific radioactivities of the I chains The figure was 31 AST U/OD_{280}/ml In order to further characterise the AST active material, an aliquot of the same dialysed L_{125_I} preparation that had been gel filtered was investigated in inhibition experiments with the rabbit sera, anti IgG AO, anti light chain, anti IgG A, and normal rabbit serum The 3 first mentioned sera gave inhibition of the AST activity of the preparation as seen from the decrease of the haemolysis inhibition zone diameters, while the normal serum did not The first antiserum was the most potent and gave virtually complete extinction, the anti-IgG A serum was the least active When the dialysed preparation L_{131_I} was similarly tried against anti IgG AO and anti light chain sera, the findings were similar When gel filtered, an AST maximum was found at R_{ribo} 0 740, the radioactivity peak had its highest value corresponding to 0 783, *i e* later in the chromatogram than the peak antibody activity

The reconstituted mixture, $H_{131_I} + L_{125_I}$ The Figure shows that L-chains and most of the H chains have been transferred from their characteristic positions in the chromatograms of free dialysed chains The heavy and light chain labels both gave a peak with a maximum at R_{ribo} = 0 558 Shouldering of heavy chain label in front of the main peak is apparent The majority of the light chains recovered from the column appeared together with the H chains in the peak while the remainder occurred at the free light chain position The calculated molar ratio H/L in the peak fraction was 1 0 and the same figure was obtained from the total radioactivity of the peak It thus appeared as if the material in the fractions from the peak was homogenous in its composition of heavy and light chains

Titration with both the plate and the micro-tube techniques showed AST activity in the fractions from the peak, following the count profile The protein concentration of the peak fraction and of the pooled material from the peak was calculated from the sum of the radioactivity counts of the heavy and

light chains, using the specific radioactivities of the isolated chain preparations The specific AST activities, obtained from these figures in conjunction with the titre values were 508 for the peak fraction and for the pooled material 501 AST U/OD $_{80}$/ml

Case EN

Specific AST activity of IgG, and of reduced alkylated and iodinated IgG (Table 3, Experiment 1)

As was the case for untreated IgG AO, the corresponding preparation gave a substantially lower specific AST activity in the micro-tube titration system than in the case of the ordinary titration technique A considerable decrease in titre was displayed by both iodinated IgG preparations The micro method also gave lower figures in this case about 50 per cent of those obtained by the other titration technique Reduction and alkylation further decreased the specific antibody activity As shown for IgG_{125_I} the values obtained with both methods agree This probably also applies to IgG_{131_I}, since almost 50 per cent haemolysis was obtained in the first titration tube

Specific AST activities of heavy and light chains, and chain mixtures (Table 3, Experiment 2) Definite titres were obtained for the heavy chain preparations the specific AST activities agreed acceptably for the 2 preparations and were less than 1 per cent of the value for reduced and alkylated IgG As no sign of activity was obtained for either of the light chain preparations and considering that the upper limits for specific activities thus found were approximately the same as the definite figures for the heavy chains the specific AST activity of the light chain preparations was probably lower than that of the heavy chains At least a tenfold potentiation of AST activity over that of free chain preparations was noted for both reconstituted chain mixtures $H_{125_I} + L_{131_I}$ and $H_{131_I} + L_{125_I}$ Recovery of antibody activity was comparatively low, approximately 9—13 per cent

Table 3 *Case EN Specific AST activity (U/OD280/ml) of IgG polypeptide chains and reconstituted chain mixtures*

Exp	Material	Specific AST activity	Potentiation	% recovery of Ab activity
1	IgG	1300 *526*		
	$IgG125_I$	572 *278*		
	$IgG125_I$ r+a	295 *287*		
	$IgG131_I$	624 304		
	$IgG131_I$ r+a	324 <*333*		
2	$H125_I$	1 92		
	$L125_I$	<. 53		
	$H131_I$	2 25		
	$L131_I$	<2 14		
	$H125_I+L131_I$	35 0	>17 4	13 2
	$H131_I+L125_I$	23 8	>10 0	9 0

Figures in italics denote results of micro tube titrations
r + a = reduced and alkylated

Characterisation of dialysed chains and chain mixture by gel filtration The same preparations as shown in Table 3, Experiment 2 were used in the gel filtration experiments

The heavy chain preparation, H_{125_I} The label appeared as a composite fraction with its maximum at R_{ribo} 0 450 No AST activity was found in the fractions

The light chain preparation L_{131_I} The radioactivity curve appeared as a slightly skewed peak with maximum at R_{ribo} 0 825 The fractions were devoid of demonstrable AST activity

The reconstituted mixture $H_{125_I} + L_{131_I}$ Most of the radioactivity recovered from the column of the heavy chain label occurred in a peak at R_{ribo} 0 562 A minor part of the recovered L label was found in a peak with a maximum at exactly the same position The main L chain label peak had its maximum at R_{ribo} 0 791 For a pool of fractions from the leading count peak the molar ratio between H and L chains was

calculated as 1 9 For the peak fraction itself the ratio was 2 0 AST titration with the plate method demonstrated antibody activity within the leading radioactivity peak, the maximum haemolysis inhibition coinciding with the peak of radioactivity Ordinary tube titration of pooled material from the peak gave no definite AST titre, but a weak inhibitory effect was discernible in the first titration tube The peak fraction gave, however, a definite AST titre The specific AST activity of the peak fraction, calculated from its AST titre, heavy and light chain radioactivity and specific radioactivities of the chains, was 148 AST U/OD_{280}/ml

Case IN

Specific ASTA activities of IgG and of reduced and alkylated, iodinated IgG (Table 4, Experiment 1)

The values obtained for untreated and radioiodinated IgG were almost identical Reduction and alkylation resulted in a minor depression of specific ASTA activity

Specific ASTA activity of heavy and light chains, chain mixtures and mixtures of chains (Table 4, Experiment 2 and 3)

No sign of ASTA activity was apparent in the light chains A definite value was obtained for H_{125_I} amounting to approx 1 5 per cent of the value for reduced and alkylated IgG The reconstituted chain mixture H_{125_I} + L_{131_I} was active and showed potentiation Potentiation was encountered for the mixture of the same chain preparations (H/L = 1/1, w/w) dialysed before mixing A similar result was obtained (Experiment 3) with the H/L ratio 1 33 (w/w)

Characterisation of dialysed chains, chain mixture and and mixture of chains by gel filtration The same preparations as given in Table 3, Experiment 2 were analysed with the exception of the mixture of dialysed chains H_{125_I} + L_{131_I} which was that of Experiment 3

The heavy chain preparation, H_{125_I} The radioactivity appeared as a principal peak at R_{ribo} = 0 288 followed by trailing and a minor complex peak at approximately 0 50

The light chain preparation, L_{131_I}, gave a radioactivity peak at R_{ribo} = 0 745 Because of the virtual absence of ASTA activity in the heavy and light chain sample scanning of the fractions for ASTA content was omitted

The reconstituted mixture H_{125_I} + L_{131_I} The heavy chain label was found in a double peak, the leading part of which (R_{ribo} = 0 386) was followed by a main peak with maximum at 0 545 The light chain label appeared in the same positions but most of the light chain label recovered, appeared in a peak of R_{ribo} = 0 773 The calculated molar ratio of heavy to light chains in the fractions around R_{ribo} = 0 545 was 0 90 and in the peak fraction it was 0 84

ASTA activity was found with plate titration coinciding with the principal heavy and

Table 4 *Case IN Specific ASTA activity (U/OD280/ml) of IgG polypeptide chains reconstituted chain mixtures and mixture of dialysed chains*

Exp	Material	Specific ASTA activity	Potentiation	% recovery of Ab activity
1	IgG	29 3		
	$IgG125_I$	27 3		
	$IgG125_I$ r+a	19 6		
	$IgG131_I$	31 3		
	$IgG131_I$ r+a	24 5		
2	$H125_I$	<0 97		
	$L131_I$	<1 15		
	$H125_I$+$L131_I$	4 11	>3 92	21 7
	* $H125_I$+$L131_I$	1 40	>1 32	
3	$H125_I$	0 42		
	$L131_I$	<0 43		
	* $H125_I$+$L131_I$	2 0	>4 71	

* mixture of dialysed chains
r + a = reduced and alkylated

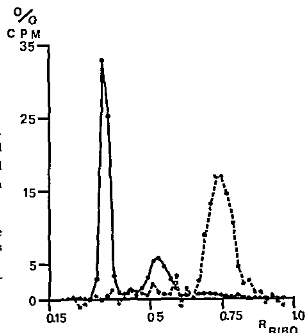


Fig 5 Text Gel filtration in Sephadex G 200 phosphate buffered saline of a mixture of H_{131_I} (IN) and L_{131_I} (IN) The chains were dialysed against tris NaCl buffer before mixing The abscissa gives the elution volume relative to that of riboflavin (R_{ribo}) The ordinate denotes the radioactivity from [1] I or [131] I as percentage of the total radioactivity recovered from the column of the respective isotope Full line signifies radioactivity from [1] I and broken line that from [131] I When free light chains (L_{131_I}) were gel filtered radioactivity was not obvious in the fractions of R_{ribo} <0 6

Table 5 *Specific AST or ASTA activities of chains and of chain mixtures at homologous recombination experiments*

Material	AST U/OD 80/ml	Potentiation	ASTA U/OD 80/ml	Potentiation
H_{125_I} (AO)	7 64			
L_{125_I} (AO)	13 5			
H_{125_I} (AO)+L_{125_I} (AO)	65 7	6 47		
H_{131_I} (IN)			<0 7-	
L_{131_I} (IN)			<0 69	
H_{131_I} (IN) +L_{131_I} (IN)			4 37	>6 18
H_{131_I} (IN) +L_{125_I} (AO)	6 64	<1 15	<0 85	
H_{125_I} (AO)+L_{131_I} (IN)	3 73	<0 85	<0 75	
H_{131_I} (AO)	11 8			
L_{131_I} (AO)	37 6			
H_{131_I} (AO)+L_{131_I} (AO)	180	7 87		
H_{125_I} (EN)	< 4 18			
L_{125_I} (EN)	< 6 62			
H_{125_I} (EN)+L_{125_I} (EN)	22 6	>4 33		
H_{125_I} (EN)+L_{131_I} (AO)	36 -	2 25> 1 96<		
H_{131_I} (AO)+L_{125_I} (EN)	15 3	2 -7> 1 59<		

In each of the - experiments all preparations were treated in parallel AST titration was carried out with the micro tube method When definite titre values were not obtained for chain preparations limits were set for the corresponding potentiation values using the upper specific antibody activity limit for the chains or zero respectively The ASTA activity of the preparations AO and EN and the AST activity for the IN samples were assumed to be zero

light chain peak The maximum ASTA value corresponded to the fraction with highest heavy and light chain radioactivity No antibody activity was evident inside the leading minor peak The findings were confirmed by tube titration using a reduced amount of staphylolysin in the assay system, the absolute titre figures are therefore uncertain

The mixture of dialysed chains ($H_{125_I} + L_{131_I}$) The heavy chain associated radioactivity emerged in a leading major peak at $R_{ribo} =$ $= 0\ 333$ containing most of the eluted [125]I activity followed by a smaller component with its maximum at 0 529 A small amount of light chain label was observed in the region of this heavy chain peak (Fig 4)

Characterisation of homologous chain mixtures

Homologous chain mixtures in formic acid were prepared with material from IgG AO, EN and GW Similarly crosses were made between chains from IgG AO and IN For comparison, autologous chain mixtures of the materials AO, EN and IN were included in the respective experiments The dialysed chain preparations and chain mixtures were analysed according to the same principles as used in connection with the autologous recombination experiments Free chain preparations and autologous chain mixtures were, however, not gel filtered

Specific antibody activity of heavy and light chains and chain mixtures (Table 5)

Compared to what had been found for the autologous reconstituted mixtures the heterologous crosses were inefficient as regards potentiation of antibody activity The most successful combinations were H_{131_I}(AO) + L_{125_I} (EN) and $H_{1\ 5_I}$ (EN) + I_{131_I} (AO) (potentiation $< 2\ 27$ $> 1\ 59$) In the crosses between chains from IgG AO and IN neither AST or ASTA activity was significantly enhanced, and the same applied to mixtures of the dialysed chains, not given in the the Table The crosses between AO and EN chains on one hand and GW chains on the other gave no potentiation (chain mixtures) H(GW) + L(EN) showed no AST activity, since the chains were without demonstrable AST activity, potentiation cold not be calculated

Characterisation of chain mixtures by gel filtration

H_{125_I} (EN) + L_{131_I} (AO) and H_{131_I} (AO) + + L_{125_I} (EN) Both preparations gave peaks containing both labels R_{ribo} was 0 543 and 0 538, respectively The rest of the light chain radioactivity was recovered in peaks of R_{ribo} 0 805 and 0 795 For both crosses, the compound heavy and light chain peaks showed AST activity at plate titration In addition, antibody activity was obvious *in the free light* chain peak from the combination H_{125} (EN) + + L_{125} (AO) at R_{ribo} 0 740 At micro tube titration definite titre values were not obtained The localisation of AST activity was, however, substantiated by the partial streptolysin inactivation discernible The specific AST activity of the main peak fractions from the 2 chromatographies was calculated as < 63 AST U/OD_{280}/ml

H_{125_I} (AO) + L_{131_I} (IN) and H_{131_I} (IN) + + L_{125_I} (AO) Major composite heavy and light chain radioactivity peaks were obtained with both preparations The respective values of R_{ribo} was 0 526 and 0 520 AST or ASTA activity was not found in the chromatographic fractions from either of the 2 heterologous mixtures

$H_{1\ 5_I}$ (AO) + L (GW) and H (GW) + L_{125_I} (AO) The heavy chain radioactivity from the first cross was apprent as a heterogenous peak with maximum at $R_{ribo} = 0\ 574$ Insignificant radioactivity was observed at the position earlier found for free AO heavy chains *i e* R_{ribo} approx 0 400 L_{125_I} (AO) from the second cross appeared as a radioactivity peak with maximum at 0 800 No radioactivity was obvious in the fractions of $R_{ribo} < 0\ 65$ AST activity was not demonstrated in the chromatographic fractions

H_{131_I} *(EN)* + *L* *(GII)* *and* *H* *(GII)* + L_{131_I}*(EN)* The findings were similar to those obtained in the crosses just described The heavy chain associated radioactivity was found in a peak with its maximum at R_{ribo} 0 535 The light chain label from the second chain mixture occurred as a peak with a R_{ribo} of 0 828 No radioactivity was found at R_{ribo} < 0 65 Because of the low AST activities of the reconstituted mixtures, titration of the chromatographic fractions was omitted

DISCUSSION

Antibody activity was demonstrated in heavy chain preparations from each of the M components AO, EN and IN and in addition in the light chain samples from IgG AO The question whether the activity was a genuine property of the respective chains or whether it was due to contaminations with other molecular species was not completely resolved

In the case of L (AO), the gel filtration experiments conclusively show that at least most of the AST activity must be caused by contaminating material There is still the possibility, however, that the light chains may possess the crucial antibody property, *i e* binding of antigen in spite of absence of haemolysis inhibition The molecular size of the AST active molecule as judged from its position in the chromatogram nearly coincides with that of Fab fragments (Chapter III) Also, the inhibition pattern with anti IgG and anti-light chain sera was similar to that obtained with the Fab preparations, *i e* marked inhibition with the anti light chain serum and less with the anti IgG serum It is conceivable that enzymatic splitting of IgG AO may have occurred during storage of the serum For the heavy chains from the case AO the gel filtration disclosed antibody activity in material greater in molecular size than IgG The figure found for contamination with light chains is high enough to explain the activity as one of reconstituted heavy and light chains If such contamination is the explanation, the reconstituted molecules must be of higher molecular size than that of IgG to fit with the gel filtration data Another possible explanation according to the electrophoretic findings is contamination with IgG This is, however, unlikely since no antibody activity corresponding to the molecular size of IgG was obvious Principally inhibition experiments with antisera may give a clue to the nature of the antibody active material in the heavy chain preparations Practically this was not possible because of the poor diffusibility of the active principle, which constituted further evidence for its high molecular nature Also in the case of heavy chains from case EN, contamination with IgG or light chains may be taken as an explanation of the observed antibody activities For heavy chains from IgG IN, *i e* H_{125_I} contamination with IgG as the sole explanation for the ASTA activity in the preparation is impossible according to the electrophoretic data This is the more so since the values obtained at electrophoresis are probably overestimated due to inclusion of radioactivity from trailing material in the calculations The figures found for light chain contamination in H(IN) were too uncertain to be taken into consideration This was because the specific radioactivities of the light chain preparations proved to be very low, giving statistically uncertain count figures together with a high multiplicative correction factor for specific radioactivity in the calculations

The autologous reconstitution experiments demonstrated in each case that heavy and light chains had recombined to aggregates of a molecular size equal to or near that of native IgG Further it was shown that reconstituted molecules were antibody active In the cases where the specific activity of the reconstituted molecules could be calculated (AO and EN) the values were lower than those for the respective reduced alkylated but not dissociated iodinated IgG preparations The propor-

light chain peak The maximum ASTA value corresponded to the fraction with highest heavy and light chain radioactivity No antibody activity was evident inside the leading minor peak The findings were confirmed by tube titration using a reduced amount of staphylolysin in the assay system, the absolute titre figures are therefore uncertain

The mixture of dialysed chains ($H_{125_I} + L_{131_I}$) The heavy chain associated radioactivity emerged in a leading major peak at R_{ribo} = = 0 333 containing most of the eluted ^{125}I activity followed by a smaller component with its maximum at 0 529 A small amount of light chain label was observed in the region of this heavy chain peak (Fig 4)

Characterisation of homologous chain mixtures

Homologous chain mixtures in formic acid were prepared with material from IgG AO, EN and GW Similarly crosses were made between chains from IgG AO and IN For comparison, autologous chain mixtures of the materials AO EN and IN were included in the respective experiments The dialysed chain preparations and chain mixtures were analysed according to the same principles as used in connection with the autologous recombination experiments Free chain preparations and autologous chain mixtures were, however, not gel filtered

Specific antibody activity of heavy and light chains and chain mixtures (Table 5)

Compared to what had been found for the autologous reconstituted mixtures the heterologous crosses were inefficient as regards potentiation of antibody activity The most successful combinations were H_{131_I}(AO) + L_{125_I} (EN) and H_{125_I} (EN) + L_{131_I} (AO) (potentiation < 2.27, > 1.59) In the crosses between chains from IgG AO and IN neither AST or ASTA activity was significantly enhanced, and the same applied to mixtures of the dialysed chains not given in the the Table The crosses between AO and EN chains on one hand and GW chains on the other gave no potentiation (chain mixtures) H(GW) + L(EN) showed no AST activity, since the chains were without demonstrable AST activity, potentiation cold not be calculated

Characterisation of chain mixtures by gel filtration

H_{125_I} (EN) + L_{131_I} (AO) *and H_{131_I} (AO) + + L_{125_I}* (EN) Both preparations gave peaks containing both labels R_{ribo} was 0 543 and 0 538, respectively The rest of the light chain radioactivity was recovered in peaks of R_{ribo} 0 805 and 0 795 For both crosses, the compound heavy and light chain peaks showed AST activity at plate titration In addition, antibody activity was obvious in the free light chain peak from the combination H_{125} (EN) + + L_{125} (AO) at R_{ribo} 0 740 At micro tube titration definite titre values were not obtained The localisation of AST activity was, however, substantiated by the partial streptolysin inactivation discernible The specific AST activity of the main peak fractions from the 2 chromatographies was calculated as < 63 AST U/OD_{280}/ml

H_{125_I} (AO) + L_{131_I} (IN) and H_{131_I} (IN) + + L_{125_I} (AO) Major composite heavy and light chain radioactivity peaks were obtained with both preparations The respective values of R_{ribo} was 0 526 and 0 520 AST or ASTA activity was not found in the chromatographic fractions from either of the 2 heterologous mixtures

H_{125_I} (AO) + L (GW) and H (GW) + L_{125_I} (AO) The heavy chain radioactivity from the first cross was apparent as a heterogenous peak with maximum at R_{ribo}=0 574 Insignificant radioactivity was observed at the position earlier found for free AO heavy chains, *i e* R_{ribo} approx 0 400 L_{125_I} (AO) from the second cross appeared as a radioactivity peak with maximum at 0 800 No radioactivity was obvious in the fractions of R_{ribo} < 0.65 AST activity was not demonstrated in the chromatographic fractions

V Some properties of the M-component-haemolysin reaction

The work presented in this Chapter concerns some aspects of the anti haemolysin activity of the M components AO, EN and IN Attempts are made to elucidate whether this activity is due to a reaction between the M components and haemolysin and if so, whether this implies a permanent binding between the reactants as is characteristic for ordinary antibodies In order to further correlate the properties of ordinary antibodies with those of the M-components comparison is made with the reactions obtained with polyclonal AST or ASTA material

MATERIALS AND METHODS

Sera The sera used were already described SE in Chapter I, AO and E N B in Chapter II
Protein preparations Pooled human anti-α-staphylolysin IgG (IgG ASTA) and pooled normal human IgG were described in Chapter I and the AST and ASTA inactive G myeloma globulin GW in Chapter IV The IgG preparations AO EN and IN were those given in Chapter IV

Iodine (^{125}I) labelling of IgG GW was carried out with the technique described in Chapter IV

Reduction and alkylation of the iodine labelled monoclonal IgG preparations the preparations IgG AO, EN and IN were the same as those used in Chapter IV and the procedure with IgG GW was that given in the same context Low molecular reactants had been removed by dialysis against at least a 100-fold volume of either tris NaCl buffer, or PBS

Radio-iodinated human serum albumin (RIHSA) was described in Chapter I The label was ^{131}I

Kinetics of streptolysin- and α staphylolysin inactivation by AST and ASTA

AST Serum SE and AO and the pooled normal human IgG preparation were diluted with PBS to an AST activity of 2 U/ml serum EN was diluted to 4 U/ml Then 0 5 ml aliquots of the preparations were added to 0 5 ml of PBS and the mixtures were incubated at 37°C for approximately 15 min in order to achieve temperature equilibration An amount (0 5 ml) of reduced (1/25 vol 10 per cent sodium pyrosulphite, 30 min, room temperature) streptolysin diluted to 1 combining U/ml was added to the tubes These were further incubated for various lengths of time after which 0 5 ml of 5 per cent sheep red cells in PBS were added After 1 hr at 37 C, 2 ml of PBS was added the tubes centrifuged and haemolysis assayed by spectrophotometry at 525 nm A control tube, containing the same dilution of streptolysin (0 5 ml) and PBS (1,0 ml) was incubated in parallel Of this 0 2 ml was transferred from the control tube at various times to other tubes containing 0 5 ml 5 per cent sheep blood and 1 3 ml of PBS together with enough pyrosulphite to make the concentration of the reducing agent in these tubes equal to that in the other haemolytic systems Haemolysis was similarly evaluated after 1 hr of incubation at 37°C after the addition of blood

ASTA Serum IN and IgG ASTA were diluted with PBS to approx 0 2 ASTA U/ml Then 0 5 ml of the dilutions were added to tubes containing 1 0 ml of PBS The tubes were incubated at 37°C for temperature equilibration together with a series of tubes with 1 4 ml of PBS After this 0 5 ml of staphylolysin (0 1 combining U/ml) in PBS

was added at various times to the tubes containing ASTA preparations and to a tube containing 1.5 ml of PBS at 37°C. Then 0.1 ml was drawn from this tube at various times and added to the tubes containing 1.4 ml of PBS. An 0.5 ml of 2 per cent rabbit red cells in PBS was immediately added to the 1.5 ml of toxin dilution and haemolysis was allowed to take place. Equal amounts of red cell suspension were added to the tubes containing ASTA preparations plus toxin, at various times after the addition of toxin to the tubes. In all systems, haemolysis was allowed to develop at 37°C for 1 hr and subsequently for 3 hrs at +5°C.

Coprecipitation of streptolysin

With the intention to study the presumed binding of streptolysin to the M-components AO and EN the following method was designed. Reduced and alkylated M component preparations are incubated with excess streptolysin in the presence of carrier IgG of low AST activity. Subsequently carrier IgG, and reduced and alkylated IgG are immunoprecipitated by addition of an anti-IgG antiserum. If stable binding of streptolysin to the M-components occurs there is the possibility of coprecipitation of the lysin. To demonstrate the presence of lysin in the precipitates these are dissolved in a denaturating agent. When this is eliminated by dialysis against PBS, some recombination of the heavy and light chains from the M component, previously dissociated by the denaturating agent, may occur. Since recombination is far from complete, as shown in Chapter IV, some lysin may remain free after dialysis and could then be assayed by its haemolytic activity.

The actual experimental procedure was as follows. Tubes with 0.5 ml of streptolysin received 100 µl of reduced, alkylated, radioiodinated IgG AO, EN, IN or GW. All additions contained the same amount of protein. IgG GW not reduced alkylated or iodine labelled in 50 µl of PBS was then added as carrier. The ratio of carrier to reduced and alkylated M-component in the mixtures was 20/1. Streptolysin was present in a calculated excess over the AST activities in the M-component preparations by a factor of at least 3. The mixtures were incubated for 30 min at 37°C. Then 0.5 ml of heat treated (56°C, 30 min) anti-IgG B serum was added. The tubes were kept at room temperature for 90 min and subsequently overnight at +5°C. The resulting precipitates were washed twice with 1 ml of cold PBS. After careful removal of the last washing, 0.5 ml of 6 M guanidinium hydrochloride in PBS and containing BSA at 0.1 g/100 ml was added. The precipitates dissolved in a few minutes and the tubes were left at room temperature for ½ hr. RIHSA in 10 µl PBS was added to each tube and the mixtures were transferred to dialysis bags; dialysis was carried out in the cold for 24 hrs against a 1000-fold volume of buffer. In order to determine the efficiency of the immunoprecipitation, the sacks with contents were then assayed for radioactivity. The contents were subsequently centrifuged and the supernatants adjusted with PBS to a constant ^{131}I (RIHSA) radioactivity per ml. This adjustment was used to correct for differences in the loss of material in the bags. The preparations received the same volume of PBSand to the mixtures was then added 1/40 volume of 10 per cent sodium pyrosulphite. Reduction of streptolysin, if any, was carried out for 30 min at room temperature. After reduction the preparations were serially diluted (dilution factor = 2) with PBS containing 0.2 per cent pyrosulphite. Haemolytic activity was assayed after the addition of 50 µl 5 per cent sheep red cells and incubation at 37°C for 60 min.

Inactivation of strepto- and staphylolysin by AST or ASTA

Variation of AST. Closely spaced dilutions of serum AO, EN and SE and of pooled normal IgG in PBS containing bovine serum albumin at a concentration of 0.1 g/100 ml (PBS—BSA) were prepared. Subsequently 0.5 ml of reduced streptolysin in PBS—BSA and con

taining 1 0 combining U/ml was added to 1 0 ml of the AST preparations The mixtures were incubated for 30 min at 37°C, after which 0 5 ml of 5 per cent sheep red cells in PBS was added to the preparations After a further 60 min incubation, 2 ml of PBS were added to the tubes After centrifugation haemolysis was evaluated by spectrophotometry of the supernatants

In some experiments (with IgG preparations AO, EN and pooled normal IgG) the procedure was varied in that streptolysin was reduced with 1/40 volume of 10 per cent sodium pyrosulphite PBS containing the same concentration of pyrosulphite but without BSA was used as diluent for reduced streptolysin and for dilution of the IgG preparations

Variation of streptolysin Serum AO, EN and SE and pooled normal IgG were diluted with PBS—BSA to 1 AST U/ml The AST content of the preparation was calculated from the results of previous titrations on other aliquots of the same material Streptolysin was reduced for 30 min with 1/25 volume of 10 per cent sodium pyrosulphite It was then diluted 5-fold This preparation was further serially diluted with PBS—BSA containing the same sodium pyrosulphite concentration as the matter diluted *i e* 0 08 per cent To 0 5 ml aliquots of the AST preparations was then added 0 5 ml of PBS—BSA and the same volume of the streptolysin dilutions Incubation of the mixtures and assay for haemolytic activity was carried out as in the experiments just described

Variation of ASTA 1 0 ml of closely spaced dilutions in PBS of IgG IN and IgG ASTA were prepared The dilutions were mixed with 0 5 ml of staphylolysin (0 1 combining U/ml) in PBS and then incubated for 30 min at 37°C After the addition of 0 5 ml of 2 per cent rabbit red cells in PBS, the samples were further incubated for 60 min at 37°C and subsequently for 3 hrs in the cold After centrifugation the supernatants were read at 525 nm for assay of haemolysis

Variation of staphylolysin 1 0 ml aliquots of IgG IN or IgG ASTA in PBS and of an ASTA activity of 0 05 U/ml were mixed with 0 5 ml of different diluions of staphylolysin in PBS After incubation for 30 min at 37°C 0 5, ml of 2 per cent rabbit red cells were added Incubation for haemolysis and its evaluation was performed as in the preceeding experiment

Simultaneous variation of AST and streptolysin 0 15 ml of dilutions of serum AO, EN and SE or pooled normal human IgG in PBS, each of an AST activity of 40 U/ml were added to 1,0 ml of streptolysin containing 8 3 combining lysin U/ml The same volumes of the same materials diluted 1/5 were mixed in centrifuge glass tubes, which also were used for the concentrated mixtures Control mixtures of 1 0 ml of streptolysin with 0 15 ml of PBS and a control mixture of the same volumes of streptolysin dilution (1/5) in PBS, and PBS were prepared The carefully stoppered tubes were incubated at 37°C for 30 min and subsequently kept for 24 hrs at +5°C. The concentrated mixtures were rapidly diluted 1/5 by addition of 4 volumes of PBS and all samples were assayed for haemolytic activity For the assay 200 100 and 50 µl from each sample was added to three tubes, containing a mixture of 1 0 ml of PBS and 0 5 ml of 5 per cent sheep red cells in PBS and sodium pyrosulphite at 0 25 per cent concentration All tubes containing sheep blood were simultaneously incubated for 60 min at 37°C then centrifuged and scanned for haemolysis by spectrophotometry at 525 nm

Test for AST activity in mixtures of AST preparations and streptolysin 5, 10 or 20 µl of serum SE AO, EN or pooled normal human IgG all diluted with PBS to 40 AST U/ml were mixed with 40 µl of streptolysin containing 9 8 combining lysin U/ml The mixtures were incubated in stoppered tubes for 30 min at 37°C Subsequently 5 µl aliquots of the mixtures were examined for AST activity with the streptolysin blood agar technique Other aliquots (25 µl) were mixed with 50 µl of PBS containing 1/25 volume of 10 per cent sodium pyrosulphite These mixtures were kept for 30 min at room temperature Then 25 µl of

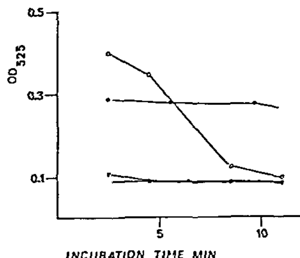


Fig 1 Kinetics of inactivation of streptolysin by IgG AO EN and pooled normal IgG Mixtures of the AST preparations and a standard amount of reduced streptolysin were incubated for various lengths of time before addition of sheep red cells The ordinate gives haemolysis as OD525 Symbols preparation AO -x x EN ▼▼ and normal IgG -o-o- Also shown is haemolysis from streptolysin without addition of AST and amounting to 13 3 per cent of the lysin dose in the other tubes (-●-●-)

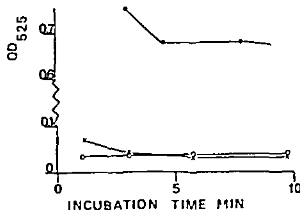


Fig 2 Kinetics of inactivation of staphylolysin by IgG IN and pooled human ASTA active IgG (IgG ASTA) Mixtures of the IgG preparations and a standard amount of staphylolysin were incubated at 37°C for various lengths of time before addition of rabbit red cells The ordinate gives haemolysis as OD525 the abscissa the time of incubation before addition of blood Symbols IgG IN x x IgG ASTA -o-o- The symbol -●●- denotes haemolysis given by staphylolysin without addition of ASTA the dose amounted to 5 per cent of that used with the ASTA preparations

5 per cent sheep red cells in PBS was added and haemolysis was allowed to develop at 37°C for 60 min Control mixtures of the AST preparations with PBS instead of streptolysin were similarly incubated and tested both for AST and haemolytic activity

Test for ASTA activity in mixtures of ASTA preparations and staphylolysin Serum In or IgG ASTA were diluted with 0 0175 M phosphate, pH 6 50 to a calculated ASTA content of 0 7 U/ml Then 2, 5, 10 or 20 µl of the preparations were added to a dilution (0 5 combining U/ml) of staphylotoxin in PBS Control preparations with PBS instead of staphylolysin were similarly prepared All mixtures were incubated for 30 min at 37°C Subsequently 5 µl aliquots were transferred to tubes, containing 25 µl of PBS plus 10 µl of 2 per cent rabbit red cells in the same buffer Haemolysis was assayed after incubation at 37°C for 60 min Other 5 µl aliquots of the mixtures were tested for ASTA activity with the rabbit blood-agar diffusion plate technique

RESULTS

Kinetics of streptolysin- and staphylolysin inactivation by AST and ASTA

AST As seen (Fig 1) the presence of IgG AO and EN resulted in a practically complete suppression of lytic activity already after incubation for 2 min before the addition of blood The effect of the pooled normal IgG seemed less pronounced In all cases, within 6 min of incubation haemolysis was lower than that from the lysin control In another experiment with serum SE, AO, EN and the normal IgG there was a sharp drop of haemolytic activity in all samples with the exception of the one with the serum SE, which showed no definite haemolysis even in the sample with the incubation time of 2 min In the other cases samples incubated for 6 min, or for longer periods gave less haemolysis than the lysin control In experiments with serum EN, diluted to an AST activity of 2 U/ml instead of 4 U/ml as in the experiments just described no definite inhibition of haemolysis was noted.

ASTA (Fig 2) For both IgG ASTA and IgG IN samples taken between 1 and 2

minutes after addition of lysin gave less haemolysis than the control lysin sample A small continuous decrease in haemolysis was evident for the sample containing IgG IN Decrease in haemolytic activity between 3 and 45 min of incubation was observed in both of the duplicate samples of the lysin control preparations

Coprecipitation of streptolysin

When the dialysis sacks were counted, their content of [125]I radioactivity was found to amount to 68—77 per cent of that originally added with the reduced and alkylated IgG preparations When the [125]I counts were normalised on the respective [131]I radioactivity figures, the values found differed by most 16 per cent of their means When streptolysin was assayed the findings were clear cut No haemolysis was noted in the tubes with IN and GW reduced and alkylated materials The AO preparation gave haemolysis in the tubes 1 2 and 3, the EN preparation in tubes 1 and 2 In a control experiment, where pyrosulphite was not included in the haemolytic system no lysis was obtained

Similar experiments were carried out using staphylolysin In some of these, excess lysis was noted in preparations containing reduced and alkylated IgG IN The difference from the control preparations with IgG AO, EN or GW was, however, difficult to assess, since haemolytic activity was obvious in all preparations

Inactivation of strepto and staphylolysin by AST or ASTA

Variation of AST (Fig 3a) The curve obtained with serum AO was less steep than that with the pooled normal IgG A kinking of the curve in the region of 50 per cent haemolysis contrasted with the continuous sharp rise of the curve resulting from the normal IgG preparation Serum EN gave a curve of quite a different form a peak of haemolysis between lower values of it from samples of both higher and lower dilution Further dilution (Fig 3 b)

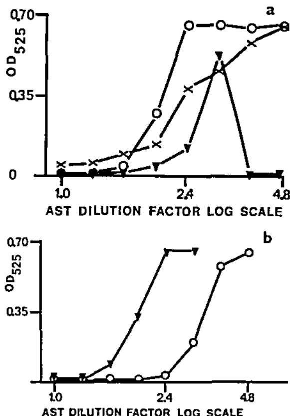


Fig 3 Inactivation of streptolysin by varying doses of a) serum AO EN and pooled normal IgG and b) serum EN and pooled normal IgG Standard amounts of reduced streptolysin and serial dilutions of the AST preparations were incubated at 37°C and the mixtures subsequently assayed for haemolytic activity The ordinate gives haemolysis as OD_{525} and the abscissa the dilution factor for the AST preparations In b) further dilutions of serum EN was employed Symbols AO x x EN ▼▼ IgG o-o-

resulted in a sigmoid curve almost congruent to that from normal IgG investigated in parallel This prozonal phenomenon was also observed in other experiments with IgG EN and with IgG AO

Depending on what part of the titration curve for serum EN that was used for determination of the AST dose giving 50 per cent haemolysis 3 different titre values were obtained These were 130 000 160 000 and 200 000 AST U/ml In one ordinary titration using doubling dilutions of IgG EN, the prozonal phenomenon was evident Two specific AST activity figures were obtained,

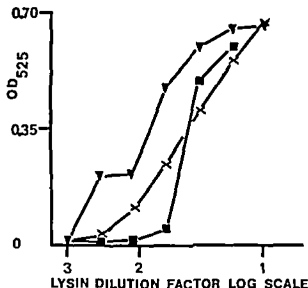


Fig 4 Reaction between varying doses of streptolysin and constant amounts of serum AO EN and SE The AST preparations were mixed with varying doses of reduced streptolysin incubated at 37°C and the mixtures assayed for haemolytic activity Ordinate haemolysis as OD525 abscissa dilution factor for streptolysin Symbols serum AO x x EN ▼▼ and SE ■ ■

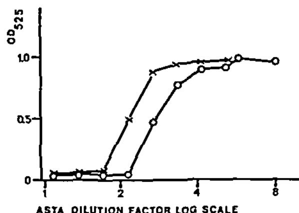


Fig 5 Inactivation of staphylolysin by varying doses of IgG IN and pooled ASTA active IgG (IgG ASTA) Standard amounts of staphylolysin and serial dilutions of the IgG preparations were incubated at 37 C and the mixtures subsequently assayed for haemolytic activity The ordinate gives haemolysis as OD525 and the abscissa the dilution factor for the ASTA preparations Symbols IgG IN x x and IgG ASTA -o-o-

i e 1200 and 4300 AST U/OD_{280}/ml At one occasion, duplicate determinations of IgG AO specific AST activity yielded the values 3200 and 3000 mean 3100 AST U/OD_{280}/ml The

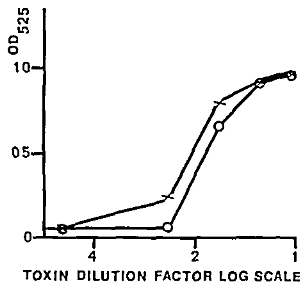


Fig 6 Reaction between varying doses of staphylolysin and constant amounts of IgG IN and pooled ASTA active IgG (IgG ASTA) The IgG preparations were mixed with varying doses of staphylolysin incubated at 37°C and the mixtures assayed for haemolytic activity Ordinate gives haemolysis as OD525 and abscissa the dilution factor for staphylolysin Symbols IgG IN x x, IgG ASTA -o-o-

AST titre of serum AO as obtained from the titration curve was 240 000 AST U/ml

Variation of streptolysin (Fig 4) Serum AO gave a curve, less steep than that from serum EN or SE investigated in the same experiment In addition, serum EN showed an irregular pattern not noticed in the other titration curves The normal pooled IgG yielded a curve similar to that of serum SE

Variation of ASTA and variation of staphylolysin (Fig 5 and 6) In both experiments employing IgG IN and IgG ASTA sigmoid curves were obtained Both materials gave similar results, *i e* the shape of the curves did not differ noticeably

AST activity in mixtures of serum AO or EN with streptolysin (Fig 7) Inhibition of haemolysis was noted in repeated experiments with the mixtures containing streptolysin and an intermediate amount of serum AO or EN When tested for haemolytic activity these mixtures gave a positive result With lower concentration of AST material no definite inhibition of haemolysis was noted Serum SE

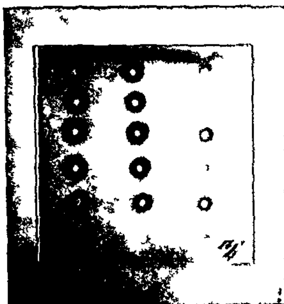


Fig 7 Streptolysin blood agar diffusion plate test for AST activity in mixtures of normal IgG serum AO or EN and streptolysin Row 1 from left to right 20 10 and 5 µl of serum AO dilution plus PBS Row 2 same serum volumes plus streptolysin Row 3 same volumes of serum EN dilution plus PBS Row 4 same serum volumes plus streptolysin Row 5 same volumes of normal IgG solution plus PBS Row 6 same volumes of IgG solution and streptolysin Haemolytic activity was present in the mixtures of streptolysin with 5 µl of IgG solution 5 and 10 µl of serum EN and with 5 20 µl of serum AO For further explanations see the Text

or the normal IgG preparation never resulted in inhibition of haemolysis in those cases where the mixtures showed haemolytic activity when tested after incubation The control preparations without lysin gave no haemolysis

ASTA activity in mixtures of serum IN or IgG ASTA and staphylolysin Inhibition of haemolysis was not noted for mixtures giving haemolytic reactions

Simultaneous variation of AST and streptolysin The results obtained in the experiments with serum AO EN SE and with normal IgG are given in Table 1 There was a definite difference in haemolytic activity between the 2 systems *i e* diluted and undiluted when the monoclonal AST preparations were tested The originally diluted system was shown to yield increased haemolysis This difference was not noted with the polyclonal preparations In these the diluted and undiluted preparations yielded the same degree of haemolysis or sometimes possibly a slightly higher haemolytic activity in the undiluted samples The same findings were obtained with the streptolysin controls As diluted and undiluted staphylolysin differed appreciably in haemolytic potency after incubation similar experiments with ASTA material were not performed

DISCUSSION

The kinetic experiments with serum AO and EN showed that the shorter the incubation time of the mixtures before addition of the red cell suspension the higher the degree of haemolysis This finding implies that constituents of the sera and streptolysin reacted resulting in the blocking of the lytic enzyme activity before addition of the red cell suspension From this one concludes that the M components AO and EN block haemolytic activity from streptolysin by reaction with the toxin For the mixtures of IgG IN and staphylolysin the findings may suggest the

Table 1 *Haemolytic activity OD 525 in mixtures of AST preparations and streptolysin at 2 different concentrations of the reactants*

Preparation	Dilution step 1	2	3
I Serum AO+lysin	0.453	0.112	0.010
Serum AO+lysin dilut	0.595	0.552	0 92
Serum EN+lysin	0.447	0.098	0.003
Serum EN+lysin dilut	0.523	0.154	0.008
Serum SE +lysin	0.591	0.380	0.034
Serum SE +lysin dilut	0.545	0 3 5	0.027
II* Serum AO+lysin	0.447	0.044	0.021
Serum AO+lysin dilut	0.633	0.562	0.208
Serum EN+lysin	0.340	0.071	0.017
Serum EN+lysin dilut	0.448	0.270	0.023
Serum SE +lysin	0.650	0.301	0.066
Serum SE +lysin dilut	0.598	0.396	0.065
III Normal IgG+lysin	1.116	0.988	0.449
Normal IgG+lysin dilut.	1.100	1.101	0.438
Normal IgG+lysin	1.095	0.865	0.422
Normal IgG+lysin dilut	1.104	0.915	0.376

Dilution step signifies the different amounts of the AST streptolysin mixtures used in the assay of haemolytic activity Roman numerals indicate sets of simultaneous determinations

* denotes assay of haemolysis in duplicate

same mechanism There was a small, but definite, drop in haemolytic activity in the mixtures taken during the first few minutes of incubation However, as heating of staphylolysin at 37°C seemed to result in some inactivation of the toxin, as shown by the toxin control, this conclusion is not unequivocally valid for the IN material If a reaction between the M-component and the staphylolysin was operative and if this reaction was rapid as suggested by the experimental findings, the slow lysis from staphylolysin should make the time course of the inactivation reaction difficult to establish In the case of serum AO EN, and SE and the pooled normal IgG the experimental results showed that at least 86 per cent of the streptolysin was inactivated within 6 min The results with IgG IN and IgG ASTA suggested that more than 98 per cent of the staphylolysin had been blocked during the first 1 5 min of incubation of the ASTA-toxin mixtures

The coprecipitation experiments demonstrated that more streptolysin activity was recovered from the precipitates containing reduced and alkylated IgG AO or EN than from those made up with reduced and alkylated IgG IN or GW, *i e* the effect was evidently correlated with the presence of AST active material Since as concluded above IgG AO and EN do react with streptolysin, the result of the coprecipitation experiment is almost certainly due to that reaction Barring the unlikely possibility that streptolysin is modified in the reaction with the M-components so as to make the lysin unspecifically stick to immunoprecipitates one can conclude that permanent binding occurs between the AST-active M components and streptolysin The findings in the experiments with IgG IN and staphylolysin were too unspecific to allow any definite conclusions

In the experiments with variation of streptolysin or AST activity in the titration systems, IgG AO and EN gave results departing from those obtained with the polyclonal AST preparations In the variation of AST content, the EN material gave rise to a prozonal phenomenon the lytic activity could be demonstrated in a narrow concentration interval when the antibody was present in excess The same finding was obtained with IgG AO It may be that haemolytic complexes between the M components and streptolysin are formed at antibody excess The prozonal phenomenon introduces ambiguity in the AST titrations of the AO and EN materials Three different titre levels may be obtained during titration *i e* depending on whether the highest haemolysis used for interpolation of the 50 per cent haemolysis end point corresponds to the ascending or the descending part of the prozonal haemolysis peak in the titration curve, or to the sigmoid part of the curve, obtained at higher dilutions of the M component Erroneously low titre values may evidently be obtained using the ordinary titration method This may explain the low recovery figures which were found (Chapter II) when the measured specific AST activities of the M components AO and EN were compared with the calculated values obtained from serum titres and contents of M component Using the highest specific activity found for IgG EN, *i e* 4300 AST U/OD $_{80}$/ml, and the concentration of M component in serum EN A, *i e* 3 8 g/100 ml (Chapter II), leads to an estimate of serum titre of 220 000 AST U/ml This is almost exactly the value obtained from the titration curve for serum EN *i e* 200 000 AST U/ml Similar calculations with the highest value of IgG AO specific activity (3100 AST U/OD$_{280}$/ml) yields a serum titre of 210 000 AST U/ml which may be compared with the result from the titration curve, *i e* 240 000 Since a prozonal phenomenon was not demonstrated in the case of IgG or serum IN the discrepancy between the specific ASTA activities directly measured, and those calculated from the serum titre and M-component concentration, respectively, remain unexplained

When the amount of streptolysin was varied in the titration system serum AO gave a less steep responce in haemolysis than noted with serum EN SE or the pooled normal IgG

Compared to polyclonal AST globulin molecules this may not necessarily indicate abnormality, since there may exist a sub population of polyclonal AST active molecules which give a dose response curve of the type encountered with AO material such molecules, together with a majority of other AST active globulins yielding a "normal polyclonal response curve, may not be able to contribute significantly to the result of the titrations The irregularity in the titration curve noted with serum EN, if outside the experimental errors may be interpreted along the same line With IgG IN there was no obvious deviation from the results encountered with the pooled normal ASTA active IgG preparation

AST activity could be demonstrated in some mixtures of streptolysin and serum AO or EN, in spite of the demonstrated lytic activity in the preparations This type of finding was not obtained with serum SE or pooled normal IgG this phenomenon was not demonstrable for mixtures of staphylolysin and ASTA active preparations, whether mono- or polyclonal The result with serum AO and EN could be given several alternative explanations

1 Free lysin and free M component may exist together in the mixture It is probable that the AST-streptolysin reaction had proceeded almost to equilibrium because the incubation time chosen (30 min) was far in excess over the time for combination suggested by the kinetic experiments If this was the case the findings imply that the affinity between streptolysin and the M components is appreciably lower than that between lysin and the polyclonal AST globulins investigated,

2 The lytic activity of the mixtures may be due to the appearance of haemolytic complexes between the M components and streptolysin In this case the complexes are either instable enough to dissociate during diffusion in the blood agar streptolysin plate resulting in liberation of free M-component or the complexes are stable and capable of binding more streptolysin, resulting in a decrease in haemolysis in the diffusion plate From the first interpretation one could again infer that the M component streptolysin binding is comparatively weak The second alternative is compatible with stable binding

When AST and streptolysin were simultaneously varied the inhibition of lytic activity was found to be markedly concentration dependent in the preparations with serum AO and EN This effect was not obtained with the polyclonal preparations Since the haemolytic activity of the streptolysin control preparations was practically unaffected by changes in concentration the findings suggest that the reaction between the M-components and streptolysin was perceptibly influenced by variations in the dilution of the system If this really was the case, the binding between M component and lysin must be comparatively weak and probably weaker than that between polyclonal AST globulin and streptolysin In the well studied BSA anti-BSA system presence of a dilution effect was shown to correlate with a low antigen antibody affinity (Farr 1958) Characteristically the early primary antibodies were of low affinity compared to the secondary response antibodies and the same has been found for anti hapten IgG antibodies (Eisen and Siskind 1964) Such findings may exemplify a general principle of differences in the quality of early and late antibodies (last ref) Thus it may be that the AST active M components AO and EN are more similar to early antibodies than the polyclonal AST preparations used for comparison

SUMMARY

With the techniques used rapid binding between the AST active M components AO and EN and streptolysin was demonstrated The findings suggested that the M components were more weakly bound to streptolysin than the polyclonal AST preparations used for comparison A prozonal phenomenon was observed in AST titrations of the M components It may explain differences found between specific AST activities of purified M components and calculated specific activities of M-components in serum

Concluding remarks

The findings of the foregoing Chapters show that the M components AO, EN and IN contain serological activity of the specificity found for the whole sera For the M components AO and EN the apparent AST activity was shown to result from binding between the M-components and streptolysin Binding of staphylolysin by the M component IN was not unequivocally proved but is considered probable in view of the collected experimental results These M components fulfil the structural requirements of antibody activity investigated for, *i e* localisation of the serological activity to the Fab (Fd) part of the molecule and formation of active composite heavy and light chain molecules at recombination of their chains

Antibody activity in the Fab fragments and ...itution of antibody active molecules ...om isolated polypeptide chains are, however, not sufficient criteria to establish the M-components as normal antibody globulins Even a pathologic structure, reacting with strepto- or staphylolysin may be localised on the Fab fragment and there is no obvious objection to reformation of an abnormal combining site structure at recombination of the polypeptide chains

Since the diagnosis of the patient AO was myeloma, *i e* a disorder of excessive proliferation of plasma cells this indicates that his M component had been produced in such cells This type of argument fails in the other cases, where this diagnosis has not been established (Waldenstrom 1967, Hallen et al 1968) The fact however that the M components consisted of both heavy and light polypeptide chains in all cases implies immunocytes as the producers of these globulins and this is postulated for the following discussion

If it is assumed that the M components AO and EN have the same specificity as the bulk of normal AST globulins the experimental findings suggest that the binding affinity of the M components is lower than that of normal AST This does not exclude these M components as normal antibodies As often demonstrated (Jerne 1951, Jerne and Avegno 1956, Farr 1958, Eisen Siskind 1964 Svehag 1965) normal antibodies display a wide variety of combining affinities According to selective antibody theories this should imply that some clones of immunocytes produce low affinity antibodies Thus there is the possibility, that the M-components AO and EN represent normal products of low affinity clones Low affinity may also be well compatible with a pathologic structure of the antibody combining site It may be surmised, that a more or less random production of configurations in a pathologic process should more often than not imply a bad fit with the antigenic configuration The finding of a G-myeloma protein with anti hapten activity (Eisen et al 1967) is of considerable interest since in this case the affinity for the antigen was directly measured The figure given for the intrinsic association constant was considerably lower than values for the average intrinsic association constant for early, low affinity rabbit antibodies against the same antigenic configuration (ε-2, 4 dinitrophenyl-L-lysin) published from the same laboratory (Eisen and Siskind 1964)

In conclusion, the M components under study have properties characteristic of ordinary antibodies This strengthens the belief, that they represent ordinary antibody molecules The deviations in behaviour from that of normal polyclonal antibodies seen in some experi

ments may reflect heterogeneity in the normal material

If low affinity against the antigen is a common feature of antibody active IgG M components, the detection of their activity may be very dependent on the sensitivity of the assay system Anti enzyme specificity of M-components should be advantageous in this respect since enzymes are possible to assay in low concentration This may explain the remarkable predominance of AST activity among the known antibody active IgG M-components, especially since AST titration is common in clinical practice

Summary

The purpose of the present investigation of 3 human sera of which 2 (AO and EN) contained an excessive antistreptolysin O (AST) activity, the 3rd (IN) an extremely elevated anti-α-staphylolysin (ASTA) activity and all IgG M components, was the following to establish whether the M-components had serological activity if so how the activity was localised in the globulin molecule Further if their polypeptide chains could be recombined to serologically active composite molecules Finally whether the M-components could bind their presumed antigens

Chapter I By modification of previously known techniques there was developed an acrylamide gel electrophoresis system, suitable for characterisation of polypeptide chain preparations A low molecular marker was found useful for standardisation of results obtained at gel filtration Microtechniques based on gel diffusion and suitable for assay of antistreptolysin or antistaphylolysin activity were designed These techniques were used in the following investigations

Chapter II Antibody activity was demonstrated in the M-components by the following criteria electrophoretic distribution of the serum IN ASTA activity inhibition of the antibody activity of the sera with antisera against IgG and against the antigenic light chain type of the respective M components but not against the respective alternative types,

correlation of the specific antibody activity of ion exchange chromatography fractions of the sera with the fractions' content of M-component

Background IgG was found to be of negligible specific antibody activity compared to that of the M-components The 2 AST active M-components were of alternative light chain antigenic types the ASTA active M component of type I The M components were of different Gm types These were in all cases compatible with the heavy chain subgroup IgG 1

Serum AO and EN AST activity was sensitive to heating at 56°C and the serum AO activity also sensitive to dialysis against low ionic strength phosphate buffer

Chapter III Proteolytic fragments were obtained from the 3 M components by gel filtration and preparative electrophoresis of papain digests Antibody activity was not demonstrated in the Fc fragments but was present in the Fab fragments For the case AO an unidentified component, possibly an Fd fragment may also contain antibody activity

Chapter IV Heavy and light chains were prepared from the radio iodine labelled M-components In all cases antibody activity was demonstrated in heavy chain preparations From the results at acrylamide gel electrophoresis of the chain preparations, contamination of the heavy chain preparations with M component or light chains could not be ruled out as the cause of the antibody activity in the heavy chain preparations An apparent antibody activity in the light chains AO was caused by contaminating material, provision ally identified as Fab fragments In autologous recombination experiments, potentiation of antibody activity was demonstrated in all cases as well as formation of antibody active composite heavy and light chain molecules Antibody active hybrid molecules were formed in the 2 homologous crosses between chains from the M components AO and EN Antibody activity could not be demonstrated in hybrid molecules created in other cross experiments among them with chains from the M-components AO and IN

Chapter V Binding between streptolysin and the M components AO and EN was demonstrated Suggestive evidence for binding of staphylolysin by the M component IN was also obtained The affinity of the AST active M components for streptolysin was judged to be comparatively low as suggested by the finding of a concentration dependence of the streptolysin AST reaction which was not obvious in the same type of reaction between lysin and polyclonal AST

A prozonal phenomenon was obvious in AST titration of serum or M-component AO and EN The phenomenon may explain discrepancies often observed between specific AST activities of purified M components and specific activities calculated for the M-components in serum

In conclusion, the M components under study have properties characteristic of ordinary antibodies This strengthens the belief, that they represent ordinary antibody molecules The deviations in behaviour from that of normal polyclonal antibodies seen in some experiments may reflect heterogeneity in the normal material

References

Badin J and Cabal N Rev Rhum *3* 17 1964

Beaumont J L Compt Rend Acad Sci *264* 185 1967

Bocci V Internat J Appl Rad Isotopes *5* 449 1964

Burstein M and Samaille J Clin Chim Acta *3* 320 1958

Christensen W N Dacie, J V Croucher B E E and Charlwood P A Brit J Haemat *3* 262 1957

Cohen S Biochem J *89* 334 1963

Cohen S and Milstein C Advances in Immunology F J Dixon Jr and H G Kunkel Editors (Academic Press New York and London) *7* 1 1967

Crumpton M J and Wilkinson J M Biochem J *88* 228 1963

Dammacco F and Clausen J Acta Med Scand *179* 755 1966

Edelman G M J Am Chem Soc *81* 3155 1959

Edelman G M Olins D E Gally J A and Zinder N D Proc Nat Acad Sci USA *50* 753 1963

Eggstein M and Kreutz F H Klin Wochschr *33* 879 1955

Eisen H N and Siskind G W Biochemistry *3* 996 1964

Eisen H N Little J R Osterland K and Simms E S Abstracts of Papers presented at the Cold Spring Harbour Symp Quant Biol June 1–6 1967 p 13

Egelund Schmidt E Mansa B and Kjems E Ugeskr Læg *130* 174 1968

Fahey J L J Immunol *91* 438 1963

Fahey J L and McKelvey E M J Immunol *94* 84 1965

Farr R S J Infect Dis *103* 239 1958

Feinstein D and Franklin E C Nature (Lond) *212* 1496 1966

Fleischman J B Porter R R and Press E M Biochem J *88* 220 1963

Fougereau M Olins D E and Edelman G M J Exp Med *120* 349 1964

Fougereau M and Edelman G M J Exp Med *121* 373 1965

Franek F and Nezlin R S Folia Microbiologica *8* 128 1963

Franklin E C Fudenberg H H and Meltzer M M Colloquium on Protides of the Biological Fluids H Peeters Editor (Elsevier Amsterdam) p 43 1963

Fudenberg H H and Kunkel H G J Exp Med *106* 689 1957

Goodman J W and Donch J J Immunochemistry *2* 351 1965

Grey H M and Kunkel H G J Exp Med *120* 253 1964

Grubb R Acta Pathol Microbiol Scand *39* 195 1956

Harboe M and Deverill J Scand J Haemat *1* 223 1964

Harboe M Deverill J and Godal H C Scand J Haematol *2* 137 1965

Hedberg H and Moritz U Acta Rheum Scand *6* 179 1960

Hsiao S and Putnam F W J Biol Chem *236* 122 1961

Hunter W M and Greenwood F C Nature (Lond) *194* 495 1962

Hallen J Waldenstrom J Winblad S and Wising P to be published in Clin Exp Immunol

Ishizaka K Ishizaka T and Hornbrook M M J Immunol *97* 75 1966

Jerne N K Acta Path Microbiol Scand Supplementum LXXXVII 1951

Jerne N K and Avegno P J Immunol *76* 200 1956

Johansson S G O and Bennich H Immunology *13* 381 1967

Killander A Killander J Philipson L and Willen R Nobel Symposia 3 Gamma Globulins J Killander Editor (Almqvist & Wiksell, Stockholm) p 359 1967

Kritzman J Kunkel, H G McCarty J and Mellors R C J Lab Clin Med *57* 905 1961

von Krogh H J Infect Dis *19* 452 1916

Kronvall, G Acta Pathol Microbiol. Scand *69* 619 1967 and personal communication.

Kunkel H G Allen J C Grey H M Martensson L and Grubb R Nature (Lond) *203* 413 1964

Kunkel H G Harvey Lectures Series 59 219 1965

Kunkel H G and Prendergast R A. Proc. Soc Exp Biol Med *122* 910 1966

LAURELL, C B LAURELL, S and SKOOG N Clin Chem. *2* 99 1956

LEGGETT BAILEY J Techniques in Protein Chemistry (Elsevier Amsterdam—London—New York) p 293 1962

LIAO S J., J Lab Clin Med *38* 648 1951

LINK H Acta Neurol. Scand *43* Suppl. 28 p 18 1967

LOWRY O H ROSEBROUGH N Y FARR A L and RANDALL, R. J., J Biol. Chem. *193* 265 1951

MAIZEL, J V JR. Science *151* 988, 1966

MANCINI G., VAERMAN J-P CARBONARA A O and HEREMANS J F., XI Colloquium on Protides of the Biological Fluids H Peeters Editor (Elsevier Amsterdam) p 370 1963

MANNIK, M and KUNKEL, H G J Exper Med *117* 213 1963

MANSA, B and KJEMS E. Acta Pathol Microbiol. Scand. *65* 303 1965

OLINS D E. and EDELMAN G M J Exp Med *119* 789 1964

OSSERMAN E. F J Immunol. *84* 93 1960

OUCHTERLONY Ö Arkiv för kemi mineralogi och geologi 26 B No *14* 1 1948

PORTER, R. R. Biochem J *73* 119 1959

PORTER, R. R and WEIR, R C., J Cell Physiol. *67* Suppl. *1* 51 1966

POULIK, M D Nature (Lond) *180* 1477 1957

ROHOLT O A. RADZIMSKI G and PRESSMAN D J Exp Med. *122* 785 1965

ROPARTZ, C. LENOIR J and RIVAT L. Nature (Lond.) *189* 586 1961

ROWE, D S and FAHEY J L. J Exp Med. *121* 171 1965

ROWE, D S and FAHEY J L. J Exp Med. *121* 185 1965

SCHEIDEGGER, J-J Int. Arch Allergy *7* 103 1955

SJÖQUIST J and VAUGHAN M H JR J Mol. Biol. *20* 527 1966

SJÖQUIST, J and ZETTERVALL O Abstracts of Papers XIth Congress of the International Society of Haematology Sydney August 1966 p 6

SVEHAG S E. Acta Pathol. Microbiol Scand *64* 103 1965

TAN M and EPSTEIN W V Science *138* 53 1963

TERRY W D and Fahey J L Science *146* 400 1964

TERRY W D and ROBERTZ M S Science *153* 1007 1966

TIPPETT L H C. Biometrika *17* 364 1925

UTSUMI S and KARUSH F Biochemistry *3* 1329 1964.

WADSWORTH C. and HANSON L. Å, Int Arch Allergy *17* 165, 1960

VAERMAN J F and HEREMANS J F Science *153* 647 1966

WALDENSTRÖM J Harvey Lectures Series *56* 211 1961

WALDENSTRÖM J Nobel Symposium 3 Gamma Globulins J Killander Editor (Almqvist & Wiksell Stockholm) p 527, 1967

WALDENSTRÖM J WINBLAD S HÄLLEN J and LIUNGMAN S Acta Med Scand *176* 619 1964.

WIEME R. J Studies on agar gel electrophoresis Techniques-Applications (Arscia Uitgaven N V, Brussel) p 442 1959

ZETTERVALL, O Acta Pathol Microbiol Scand *69* 616, 1967 a

ZETTERVALL, O Nobel Symposium 3, Gamma Globulins J Killander Editor (Almqvist & Wiksell Stockholm) p 349 1967 b

ZETTERVALL, O SJÖQUIST J WALDENSTRÖM J and WINBLAD S Clin. Exp Immunol. *1* 213 1966

ZIKAN J and STERZL, J Nature (Lond) *214* 1225 1967

Acta Medica Scandinavica

Supplementum 493

The Stockholm Prospective Study 1

The Initial Values for Plasma Lipids

By Lars A Carlson and Sven Lindstedt

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv*, was founded in 1869 by Professor Axel Key, MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*.

Acta Medica Scandinavica

publishes papers on general medicine from Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German Acta Medica Scandinavica is published on a non-profit basis

to Acta Medica Scandinavica (two volumes of six numbers each a year) include free supplements to the current volumes During the past few years an average number of 17 supplements has been issued per annum

Subscription Rates

Per annum = two volumes
In Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Sw kr 120, incl postage
Other countries Sw kr 140, incl postage

Chief Editor

Associate Professor Birger Strandell, MD
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Biblioteksgatan 11
S-111 46 Stockholm, Sweden

Subscription and Distribution

Almqvist & Wiksell
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1, Sweden

Printers

Almqvist & Wiksells Boktryckeri AB
Box 616
S-751 25 Uppsala 1, Sweden

THE STOCKHOLM PROSPECTIVE STUDY 1

The Initial Values for Plasma Lipids

THE STOCKHOLM PROSPECTIVE STUDY

1

The Initial Values for Plasma Lipids

By LARS A CARLSON and SVEN LINDSTEDT

from

The Departments of Internal Medicine and Clinical Chemistry,
Karolinska sjukhuset, King Gustaf V Research Institute,
The Chemistry Department 1, Karolinska institutet and
Företagens hälsokontroll, Stockholm, Sweden

Supported by a grant from Folksam Insurance Company, Stockholm, Sweden

Printed in Sweden
Stockholm 1968 Kungl. Boktryckeriet P. A. Norstedt & Söner

CONTENTS

INTRODUCTION

The association between high levels of cholesterol in blood and the presence of coronary heart disease is well recognized and has been studied frequently during the last 20 years (for reviews see 97, 59, 4) Recently the Framingham prospective study has definitely shown that there is also a direct correlation between the serum cholesterol concentration and future development of coronary heart disease (30 57, 56) Similar results have been obtained in other prospective studies (34, 35, 88, 67, 114)

The concentration of triglycerides in serum is frequently elevated in patients with coronary heart disease (6 22 11, 50) The concentration of cholesterol and triglycerides in plasma may vary independently of each other, consequently, a patient may have one of these lipid fractions elevated and the other within normal limits (22, 50 49) In fact elevated triglyceride levels have been reported to occur at least as frequently as elevated cholesterol levels in retrospective studies of subjects with coronary heart disease (6 22 50) When this study was started no prospective study had been carried out in which the concentrations of both cholesterol and triglycerides had been determined and related to the development of coronary heart disease

In the present study, 'The Stockholm Prospective Study', the serum concentrations of total cholesterol and triglycerides will be related to the development of coronary heart disease in different age classes The appearance of this disease will be studied five or more years after the initial examination The present report describes the initial values for serum lipids in the 6464 subjects included in the study These lipid values have been related to a number of factors which are known to be associated with the presence of coronary heart disease, i e hereditary factors, systolic and diastolic blood pressures, type of employment, weight height relation, smoking habits and physical activity

METHODS

Subjects

The subjects studied in this work were examined at Foretagens Halsokontroll in Stockholm from August 1st, 1961 to July 30th 1962 The Foretagens Halsokontroll is a health control station, run cooperatively by a number of companies which yearly offer a free medical examination to their employees The serum lipid values were determined in 3624 men and in 2840 women This represents 77 1 % of all subjects examined at Företagens Hälsokontroll during the time of this study

Clinical examination

The medical examination was carried out by the physicians at Företagens Hälsokontroll who followed a special questionnaire when obtaining the case histories This questionnaire had been designed for the present study with special emphasis on diseases and habits of potential interest in relation to blood lipids The information was coded and transferred to punch cards Weight (without clothes) and height (without shoes for women) were recorded for each subject A weight height index was calculated as weight in kg/(height in cm—100) (38) Systolic and diastolic blood pressures were obtained at the end of the examination with the use of a mercury manometer The diastolic blood pressure was recorded at the disappearance of the 5th Korotkow sound A resting electrocardiogram (ECG) was recorded with standard leads and five chest leads (CR$_1$ CR$_2$ CR$_4$ CR$_5$ and CR$_7$) A chest radiogram was also obtained for each subject and evaluated by a radiologist at Företagens Hälsokontroll

Analytic procedures

Hemoglobin was determined as oxyhemiglobin 16 0 g per 100 ml was set equal to 100 % On the basis of a determination of hemoglobin concentration in 2014 men and 1402 women between June and December 1961 the following normal values were used for our classification of the material (see below group 1 b) men 80 to 105 %, women 70 to 105 % The hemoglobin concentration in 94 8 % of the men and in 96 7 % of the women fell within these limits The erythrocyte sedimentation rate (E S R) was determined with standard methods E S R less than 10 mm per hour for men and less than 20 mm for women was considered as normal The urine was tested for the

presence of protein and glucose with Albustix® and Clinistix® (Ames Company)

Lipid analysis

Blood was drawn by venipuncture in the morning after fasting over night, allowed to clot at room temperature and the serum separated by centrifugation Cholesterol was determined with the Tschugaeff reaction (110, 48) and triglycerides according to Carlson (23)

Reagents

Diethyl ether methanol chloroform and acetyl chloride (E Merck A. G Darmstadt, Germany) were of analytical grade For unknown reasons no color developed in the Tschugaeff procedure when acetyl chloride from a few other manufacturers was used

Sodium chloride solution Nine g of sodium chloride was dissolved in 1 l of distilled water

Ethanolic potassium hydroxide Fifty ml of a 25 % (w v) solution of potassium hydroxide was diluted with 950 ml of spectroscopic grade absolute ethanol The solution was stored in the refrigerator and prepared fresh each month

Silicic acid Silicic acid 100 mesh (Mallinckrodt Chemical Works St Louis Mo USA) was activated at 100 C in an oven for 12 hours

Sulfuric acid Concentrated sulfuric acid 350 g was diluted to 1 l with distilled water

Sodium arsenite 0.2 M Four g of arsenic trioxide E Merck A G) was brought into solution by heating with 1 8 g of sodium hydroxide in 25 ml of distilled water The solution was diluted to 200 ml with distilled water after cooling

Sodium periodate 0.2 M Sodium metaperiodate (0 85 g) (Riedel—de Haen A. G., Seelze Hannover Germany) was dissolved in 200 ml of distilled water The solution may also be prepared from metaperiodic acid (The G Frederick Smith Chemical Co, Columbus Ohio, USA) by dissolving 0 77 g in about 100 ml of water, neutralizing with 0 2 M sodium hydroxide and diluting to a final volume of 200 ml with distilled water

Chromotropic acid One g of chromotropic acid (4.5 dihydroxy 2 7 naphtalenedisulfonic acid disodium salt, E Merck A. G, analytical grade) was dissolved in 100 ml of distilled water filtered and mixed into a cold mixture of 1300 ml concentrated sulfuric acid and 750 ml of distilled water The solution was kept in dark bottles

Zinc chloride Zinc chloride 130 75 g (E. Merck A G, analytical grade) was dissolved in 500 ml of glacial acetic acid

Tschugaeff reagent The reagent was prepared by mixing 1000 ml of chloroform, 500 ml of zinc chloride in glacial acetic acid and 500 ml of acetyl chloride It could be kept for at least 2 weeks

Triglyceride standard Recrystallized tripalmitine (0 807 g) was dissolved in 1000 ml of chloroform. Working solutions containing 0 2 mmole per l and 0 4 mmole per l were prepared by dilution with chloroform and used daily for determination of a standard curve

Cholesterol standard Four hundred mg of cholesterol recrystallized from acetone and water (mp 148° C) was dissolved in 400 ml of chloroform Working solutions containing 20 mg per 100 ml and 40 mg per 100 ml were prepared by dilution with chloroform and used daily for the determination of a standard curve

Procedure

Extraction One ml of serum was pipetted into test tubes (30 mm×90 mm) and 5 ml of methanol was added with gentle swirling followed by 10 0 ml of chloroform and 15 ml of sodium chloride solution. The following day about 7 ml of the chloroform bottom layer was transferred with a hypodermic syringe to a test tube (16 mm×100 mm) with a ground glass stopper (N S 12) Silicic acid (0.5 g) was added the test tube shaken vigorously and centrifuged with the stopper in place

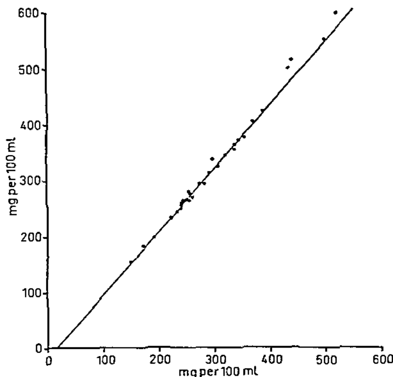


Fig 1 Values for serum cholesterol obtained by the method used in this study (Y axis) and by the Sperry Webb method (X axis) on the same randomly selected serum samples

Triglyceride determination Duplicates of 0 5 ml of the chloroform solution were pipetted into test tubes of the same type as used for the silicic acid treatment 1 ml of potassium hydroxide in ethanol was added and the stoppered tubes left in a water bath kept at 60 C for 30 min. At this stage duplicate standards and blanks of chloroform were introduced into the procedure After cooling to room temperature 0 8 ml of sulfuric acid and 4 ml of ethyl ether were added and the tubes shaken The ether layer was removed by suction with a capillary tube connected to the water pump and the tubes left without s opper for about 1 hour From each tube 0 3 ml was transferred to test tubes (10 mm×150 mm) which had been calibrated for use as cuvettes in the spectrophotometer 0 1 ml of sodium periodate was added followed after 10 minutes by 0 1 ml of sodium arsenite Five minutes later 2.5 ml of chromotropic acid were mixed into the solution and the test tubes placed in a boiling water bath for 30 minutes After cooling to room temperature the tubes were wiped dry and the absorbancy was read at 570 nm in a Beckman B spectrophotometer fitted with a holder for cylindrical cuvettes

Cholesterol determination From the silicic acid treated chloroform solution duplicates of 0 2 ml were transferred to calibrated test tubes Duplicates of 0 2 ml of standards and chloroform blanks were also pipetted into test tubes Five ml of the Tschugaeff reagent were added and the test tubes left for 15 min

in a water bath at 60° C. The test tubes were cooled, wiped dry and the absorbance was then read at 528 nm in a Beckman B spectrophotometer.

Analytical errors

The methodological error was calculated on duplicate analyses as

$$\sqrt{\frac{\sum d^2}{2N}}$$

in which d is the difference between duplicates and N the number of samples. The error of the method was then found to be 0.11 to 0.13 mmole per l in the triglyceride assay and 6—8 mg per 100 ml in the cholesterol assay.

Comparison with other cholesterol methods

The values for cholesterol concentration obtained by the present technique were on several occasions compared with results obtained by the Sperry and Webb method (102). Fig. 1 shows a comparison of the values obtained on 50 samples analyzed by the two methods. The equation for the regression line is

$$y = 1.15x - 19 \quad \begin{matrix} x & \text{Sperry and Webb's method} \\ y & \text{Tschugaeff's method} \end{matrix}$$

In order to facilitate the comparison of the figures for serum cholesterol concentration obtained in the present study with values obtained in other population studies it was considered valuable to compare the analytical results with results obtained by an independent laboratory. Professor Flaminio Fidanza at the Institute of human physiology of the University of Naples generously offered to perform analyses by the method of Abell et al. (1) as modified by Anderson and Keys (10), a method which has been used in most of the population studies carried out by Keys and coworkers. Samples were therefore on several occasions sent in duplicate to Naples for cholesterol analysis. Fig. 2 shows the comparison of results obtained on 57 different samples analyzed by our laboratory and that of Dr. Fidanza. The equation for the regression line is

$$y = 1.15x - 39 \quad \begin{matrix} x, & \text{Anderson and Keys method} \\ y, & \text{Tschugaeff's method} \end{matrix}$$

Classification of subjects

For the purpose of this report the subjects were successively classified into ten groups in such a way that a subject which had been placed in one group did not appear in any of the other groups. The criteria used for the classification were as follows.

Group 1. a) Subject had received a clinical diagnosis by the examining physician. The World Health Organization (WHO) classification code was used to record the diagnoses, which are listed in Table A 1[1] together with the number of subjects referred to each diagnostic number. Subjects with minor and—in relation to this study—unimportant diseases were not referred to group 1. Subjects with hypertension, obesity and coronary heart disease were referred to separate groups (see below). b) Hemoglobin concentration outside normal limits. c) E.S.R. above normal limits. d) Presence of glucose or protein in the urine. e) Chest radiogram showing pathological processes in the lungs or in the pleura which were not due to healed tuberculosis. f) Pregnant women. g) Dietary abnormalities such as subjects on active weight reduction or vegetarians.

Group 2. Systolic blood pressure above 170 mm Hg and/or diastolic blood pressure above 100 mm Hg or subjects with WHO code number 444 (hypertension).

[1] Tables A 1, A 2 etc. refer to Tables 1, 2 etc. in the Appendix.

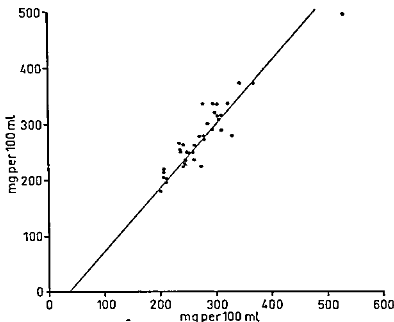


Fig 2 Values for serum-cholesterol obtained by the method used in this study (Y axis) and by the filter paper disc method by Dr Fidanza Naples (X axis) on the same, randomly selected serum samples

Group 3 Subjects with history or signs of cardiac disease other than coronary heart disease, i e valvular disease myocarditis etc

Group 4 Subjects given the clinical diagnosis obesity by the examining physician This diagnosis was used when the weight height index was 1 1 or more for subjects with a height of less than 180 cm, and 1 05 or more for subjects with a height above 180 cm (38)

Group 5 Subjects given the diagnosis coronary heart disease by the examining physician on the basis of a case history suggestive of angina pectoris or myocardial infarction

Group 6 Subjects with suspected pathological or pathological ECG

Group 7 Subjects for which no ECG was obtained

Group 8 Subjects with family history of cerebral vascular disease i e subjects whose parents suffered from cerebral vascular disease which had been diagnosed before the age of 65 years

Group 9 Subjects with family history of coronary disease i e subjects whose parents suffered from coronary heart disease which had been diagnosed before the age of 65 years

Group 10 Healthy subjects i e all subjects remaining after the exclusion of those referred to group 1 to 9

Statistical methods

Statistical calculations were performed as recommended by Snedecor (101)

RESULTS

Composition of material

The total number of subjects studied was 6464 of which 3624 were men and 2840 were women. The numbers of men and women in each group as well as the age distributions are given in Fig. 3 and in Tables A 2 and A 3. Table A 1 lists the number of subjects in group 1 in each of the WHO diagnosis classes. Group 1, containing various diagnoses, and group 10, the "healthy" group were the largest. Group 10 comprised one third of the entire material. The figure 33 % is quite comparable to the results from the Stockholm City Health Survey of 1954 (38) in which 20 per cent of the persons examined had no morbid condition or abnormality, considering the fact that group 10 includes also persons with diagnoses considered as non essential in relation to the serum lipid values. Conditions considered relevant to serum lipids were listed in four major categories: a) Conditions known to be associated with serum lipid abnormalities (diabetes, hyperthyroidism etc.) b) Conditions with more or less general effects on the person's health (cancer, infectious diseases, anemia etc.) c) Conditions in which lipid abnormalities may occur (cardiovascular disease, obesity, heredity for cardiovascular disease etc.) d) Conditions in which the subjects might take medicine (e.g. salicylate, antihistamines) or otherwise fairly regularly be exposed to influences possibly having an effect on serum lipids (e.g. arthritis, vegetarian habits). Examples of diagnoses accepted as non-essential are: Cerumen in the external auditory canal, refraction defects, inguinal hernia, varicose veins without complications, dental caries, seborrhea.

The results will be described first for group 10 comprising apparently healthy subjects. This forms the basis for comparison with groups 1 to 9.

Frequency distribution of values for serum cholesterol and serum triglyceride concentration in group 10

The frequency distributions of values for serum cholesterol concentration, serum triglyceride concentration and the logarithms of serum triglyceride concentration in the different age classes are given for group 10 in Fig. 4 to 6 and in Tables A 4 to A 9. The frequency distributions were tested for skewness as indicated in Fig. 4 to 6. Tables A 10 to A 12 give the statistical index of skewness in the frequency distribution (g) and its standard

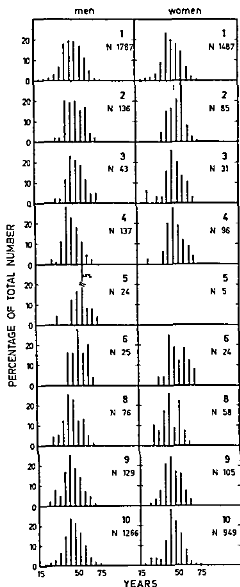


Fig 3 Age distribution of subjects in group 1 to 10 Class interval 5 years

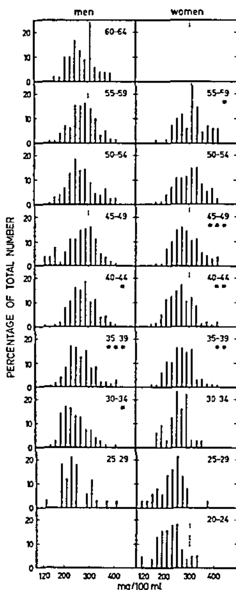


Fig 4 Distribution of values for serum-cholesterol concentration in the different age classes of healthy men and women (group 10) Class interval 20 mg per 100 ml * ** and *** indicate that the distribution is significantly skewed at the 5 1 and 0 1 per cent level

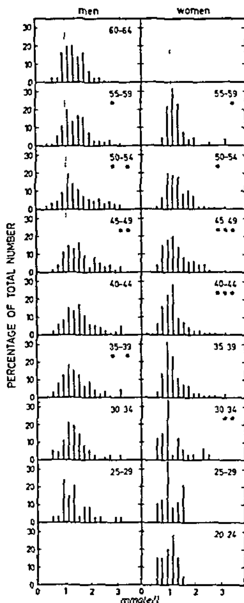


Fig 5 Distribution of values for serum triglyceride concentration in the different age classes of healthy *men and women (group 10)* Class interval 0 2 mmole per l *, ** and *** see figure 4

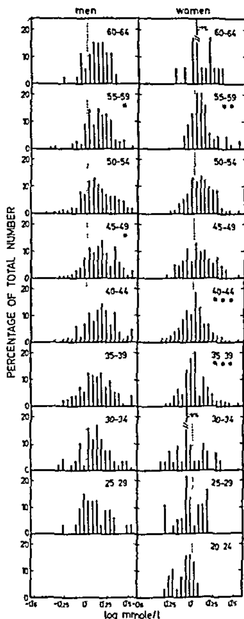


Fig 6 Distribution of values for the logarithm of serum triglyceride concentration in the different age classes of healthy men and women (group 10) Class interval 0 05 log mmole per l *, ** and *** see figure 4

TABLE I Per cent of healthy subjects (group 10) within each age class with serum-cholesterol concentration above 400 mg per 100 ml or serum triglyceride concentration above 3 0 mmole per l

Age	Serum cholesterol concentration above 400 mg per 100 ml		Serum triglyceride concentration above 3 0 mmole per l	
—	Men	Women	Men	Women
25—29	3 0	—	3 0	—
30—34	1 4	—	2 8	—
35—39	2 8	0 9	3 9	0 9
40—44	1 0	1 9	5 5	0 4
45—49	1 9	3 4	3 7	0 5
50—54	2 5	2 6	2 0	0 7
55—59	1 6	5 8	0 8	2 9
60—64	—	16 7	—	—
65—69				

error (s_g) As seen in Fig 4 the distribution of values for serum cholesterol concentration was significantly skewed (g=0 39 to 1 24) in several age classes A more pronounced skewness was found in the distribution of the values for serum triglyceride concentration (g=0 99 to 5 02) (Fig 5) In all cases the mean value was greater than the median The skewness was reduced after the transformation of the triglyceride concentration values to their logarithms, but was still present in three age classes of women and two of men (Fig 6) Therefore, both the triglyceride concentrations and the logarithms of the triglyceride concentrations have been used in the statistical calculations in this work

In order to give a rough estimate of the frequency of *markedly* elevated plasma levels we have listed in Table I the per cent of subjects in each age class with cholesterol concentration above 400 mg per 100 ml or triglyceride concentrations above 3 mmole per l About 2 per cent of the apparently healthy men had cholesterol values above 400 The corresponding proportion of women increased with age and was higher than that for men above 40 years More men than women about 3 per cent against 1 per cent had significantly elevated triglyceride levels

Age, sex and concentrations of serum cholesterol and serum triglycerides in group 10

Fig 7 shows the relation between cholesterol concentration and age in group 10 The mean values for serum cholesterol concentration with their standard deviations and standard errors are given for the different age classes in Table A 10 It is apparent that in the age class 15 to 19 years, women had a significantly higher serum cholesterol concentration than men At the age of 20

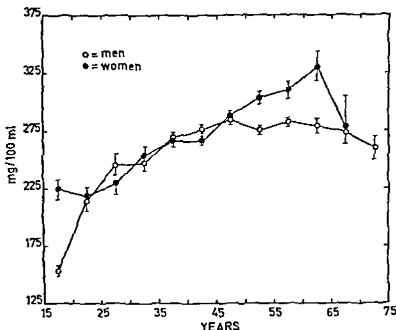


Fig 7 Serum cholesterol concentration (mean value ± standard error of the mean) and age in healthy men and women (group 10)

to 24 years the level of serum cholesterol was the same (about 250 mg per 100 ml) in men and women and it then increased in a parallel manner for both sexes to the age of 45 to 49 years From this age the serum cholesterol concentration continued to increase in women whereas it levelled off in men which resulted in a highly significant difference between men and women at the ages 50 to 64 years The standard deviation for the serum cholesterol concentration was about 50 mg per 100 ml for both men and women

Fig 8 shows the relation between serum triglyceride concentration and age in group 10 The mean values for the serum triglyceride concentration and for the logarithm of serum triglyceride concentration with their standard deviations and standard errors are given in Tables A 11 and A 12 In both men and women the serum triglyceride concentration increased from 0 8 to 0 9 mmole per l at 15 years to 1 58 mole per l and 1 23 mmole per l, respectively, at the age of 50 years, whereafter the triglyceride concentration declined significantly in men Between 25 and 55 years the serum triglyceride concentration in men was significantly higher (0 2 to 0 4 mmole per l) than in women The standard deviation for the serum triglyceride concentration was 0 5 to 0 6 mmole per l (Tables A 13 and A 14)

There was no simple linear relation between age and the lipid values An attempt was therefore made to describe

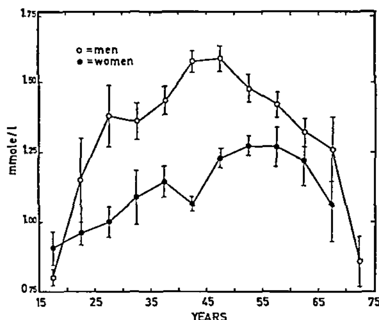


Fig 8 Serum triglyceride concentration (mean value ± standard error of the mean) and age in healthy men and women (group 10)

the relation by polynomials of higher degree The data of the statistical analysis of variance and regression are given in Tables II—VII

The relation between age (x, years) and cholesterol level (y, mg/100 ml) in men was best described by a second degree equation

$$y = 78\,5 + 7\,81\,x - 0\,0745\,x^2$$

The maximal cholesterol concentration of 284 mg per 100 ml was reached at an age of 52 5 years

The statistical analysis of the relation between age and cholesterol concentration in women showed a dominant linear component and the addition of terms

TABLE II Analysis of variance and regression of cholesterol on age for men in group 10

Source of variation	d f	Sum of squares	Mean square
Linear regression	1	94 731	94 731
Quadratic regression	1	103 004	103 004
Deviation from quadratic regression	9	33 625	3 736
Total regression	11	231 360	21 033
Error	1254	3 426 693	2 733
Total	1265	3 658 053	

TABLE III Analysis of variance and regression of cholesterol on age for women in group 10

Source of variation	d f	Sum of squares	Mean square
Linear regression	1	544 609	544 609
Deviation from linear regression	10	60 700	6 070
Total regression	11	605 309	55 028
Error	937	2 698 880	2 880
Total	948	3 304 189	

TABLE IV Analysis of variance and regression of triglycerides on age for men in group 10

Source of variation	d f	Sum of squares	Mean square
Linear regression	1	418	418
Quadratic regression	1	125 181	125 181
Deviation from quadratic regression	9	24 935	2 711
Total regression	11	150 538	13 685
Error	1254	5 571 600	4 443
Total	1265	5 722 138	

TABLE V Analysis of variance and regression of log triglycerides on age for men in group 10

Source of variation	d f	Sum of squares	Mean square
Linear regression	1	0 002	0 002
Quadratic regression	1	1 060	1 060
Deviation from quadratic regression	9	0 253	0 028
Total regression	11	1 315	0 120
Error	1254	38 622	0 031
Total	1265	39 937	

of second and third degree did not result in any improvement The linear equation was
$y = 161\ 0 + 2\ 63\ x$

The relation between age and triglyceride (y, mmole/l) in men was well described by a second degree polynomial in which most of the variance between

TABLE VI Analysis of variance and regression of triglycerides on age for women in group 10

Source of variation	d f	Sum of squares	Mean square
Linear regression	1	65 040	65 040
Quadratic regression	1	136	136
Cubic regression	1	3 580	3 580
Deviation from cubic regression	8	27 396	3 425
Total regression	11	96 152	8 741
Error	937	2 078 116	2 218
Total	948	2 174 268	

TABLE VII Analysis of variance and regression of log triglycerides on age for women in group 10

Source of variation	d f	Sum of squares	Mean square
Linear regression	1	1 033	1 033
Quadratic regression	1	0 004	0 004
Cubic regression	1	0 065	0 065
Deviation from cubic regression	8	0 357	0 045
Total regression	11	1 459	0 133
Error	937	23 835	0 025
Total	948	25 294	

the age classes could be related to the quadratic component

$y = -0\ 163 + 0\ 0751\ x - 0\ 000821\ x^2$

There was a maximum of 1 56 mmole/l at 45 7 years of age

When the analysis of variance and regression was carried out on the logarithms of the triglyceride values (y log mmole/l) a second degree polynomial was also found to give a very good fit of the experimental data

$y = 1\ 64 + 0\ 02212\ x - 0\ 000239\ x^2$

The relation between age and triglyceride concentration in women was more difficult to analyze than in men There was a major linear component with a deviation at higher ages but the introduction of a quadratic term did not result in an improvement The addition of a cubic term resulted in a better fit but there remained a considerable residual variance The possible equations for the relation between age and triglyceride concentration would thus be

$y = 0\ 753 + 0\ 00910\ x$

$y = 1\ 443 - 0\ 0482\ x + 0\ 00147\ x^2 - 0\ 0000119\ x^3$

The use of logarithmic values did not make it easier to fit a curve to the observed data and either a linear or cubic equation could be considered

$y = 1\ 87 + 0\ 0036\ x$

$y = 2\ 22 + 0\ 024x + 0\ 000670\ x^2 - 0\ 00000521\ x^3$

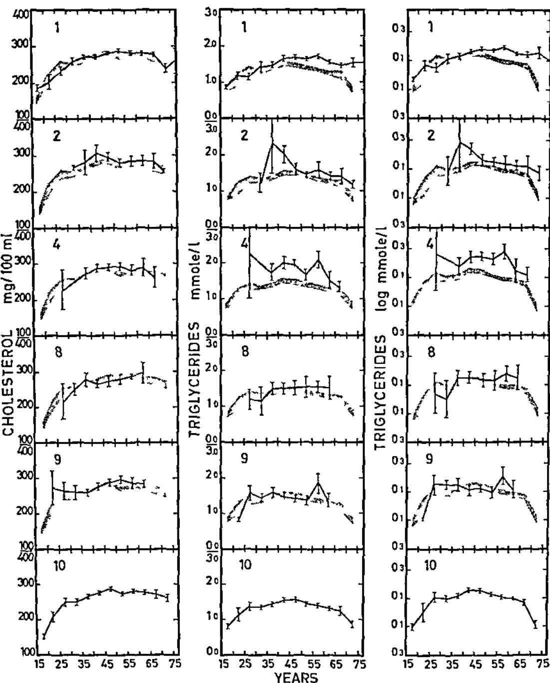


Fig 9 Serum cholesterol and serum triglyceride concentration and log serum triglyceride concentration (mean value ± standard error of the mean) in groups 1 2 4 8 9 and 10 for men of age 15—75 years Shaded area is mean value ± standard error of the mean for healthy subjects (group 10)

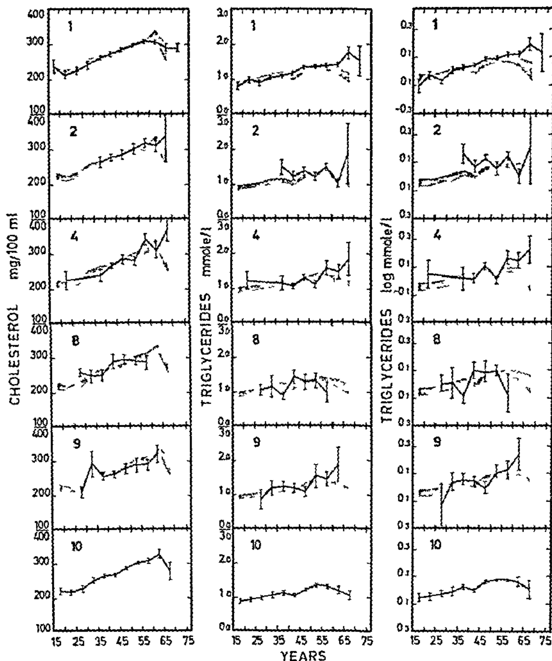


Fig 10 Serum cholesterol and serum triglyceride concentration and log serum triglyceride concentration (mean value ± standard error of the mean) in groups 1 2 4 8 9 and 10 for women of age 15—75 years Shaded area is mean value ± standard error of the mean for healthy subjects (group 10)

Concentrations of serum cholesterol and serum triglycerides in groups 1 to 9

Fig 9 and 10 show the relation between concentrations of serum lipids and age in groups 1, 2, 4 8, 9 and 10 The concentrations in all age classes in these groups are listed in Tables A 15 to A 30 It is seen from the figures that no significant differences existed between the different groups in the values for serum cholesterol concentration for either men or women The serum triglyceride concentration in men was significantly higher in group 1 than in group 10 from the age of 45 years A similar tendency, although less marked, was noted for women In the group of obese males (group 4) the serum triglyceride concentration was 0 3 to 0 5 mmole per 1 higher than in the healthy group (group 10) Obese women, however, did not have a significantly higher serum triglyceride concentration than healthy women There were no systematic differences in serum triglyceride concentration between the remaining groups and the healthy group

Age, sex and blood pressure

Fig 11 and Tables A 31 to A 32 show the relation between systolic and diastolic blood pressure and age in group 10 for men and women It should be kept in mind that subjects with a clinical diagnosis of hypertension and/or blood pressure above 170/100 had been referred to group 2 Men in the younger age classes had a systolic blood pressure around 130 mm Hg which showed a significant increase from 15 to 74 years with 0 39 ± 0 04 mm Hg per year The systolic blood pressure in women increased with 0 73 ± 0 05 mm Hg per year from an initial value of about 120 mm Hg in the younger age classes The diastolic blood pressure in young men was about 80 mm Hg and increased significantly to the age of 74 with 0 19 ± 0 02 mm Hg per year whereas the young women had a diastolic pressure of 75 mm Hg which increased with 0 35 ± 0 03 mm Hg per year The differences between blood pressures of men and women in the younger ages and the differences in regression coefficients were highly significant The figures for blood pressure and age found here are remarkably similar to previously published data from Scandinavia (16 87)

The frequency distributions of systolic and diastolic blood pressure were tested for skewness (Tables A 31 and A 32) and the distributions of the systolic blood pressures were found to be skewed for both men and women in most age classes The diastolic blood pressure was normally distributed in men whereas skewness was found for women in two age classes

The blood pressures of subjects referred to groups 1 to 6, 8 and 9 are given in Tables A 35 to A 42 In group 2 (hypertension) the systolic blood pressure was 30 to 50 mm Hg above and the diastolic pressure about 25 mm Hg above the values in group 10 In groups 1 and 4 a slightly elevated pressure (systolic pressure 5 to 10 mm Hg, diastolic pressure 2 to 5 mm Hg) was noted in some age classes In the other groups no differences from the healthy material were found

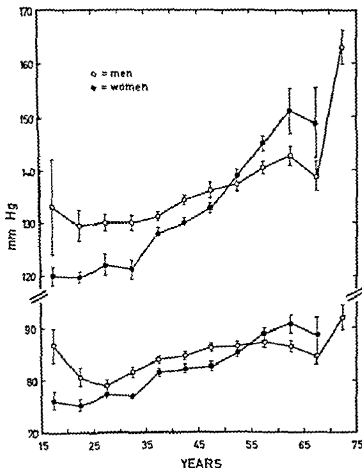


Fig 11 Systolic and diastolic blood pressure and age in healthy men and women (group 10) (mean value ± standard error of the mean)

Age, sex and weight height index

Fig 12 and Tables A 43 and A 44 show the relation between the weight height index and age for men and women in group 10 Subjects classified as obese had been referred to group 4 For non obese men this index increased from 0 877 at 20 to 24 years to 0 950 at the age of 49 which represents an 8 per cent increase in body weight After the age of 49 years the index remained fairly constant to the age of 64 years and then declined to 0 912 at 74 years For women the index increased from 0 847 at 20 to 24 years to 0 998 at the age of 64 which represents an 18 per cent increase in body weight The index had about the same value for both men and women up to the age of 50 whereafter the index was higher in women As shown in Table

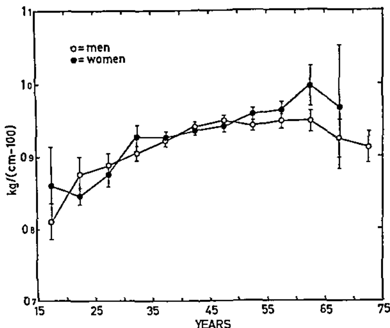


Fig 12 Weight height index (mean value ± standard error of the mean) and age in healthy men and women (group 10)

A 44 there was a highly significant regression of the weight height index on age for both men and women The distribution of the index showed significant negative skewness in four age classes of men and in one age class of women and positive skewness for women in the age 35 to 39 years

The values for the weight height index for groups 1 to 6, 8 and 9 are given in Tables A 45 to A 52 In the hypertensive group (group 2) the weight height index was about 1 05 as compared to about 0 95 in the healthy group However these values cannot be directly compared as hypertensive subjects who were also obese had not been excluded from group 2 In the obese group (group 4) the index was around 1 15 for men and 1 25 for women

Environmental factors, plasma lipids, blood pressure and weight height index in group 10

Group 10 was divided into subgroups to study the levels of lipids, blood pressures and weight height index in the subgroups As these variables all vary with age we have maintained the age classes within the subgroups, which means that many classes will be small However, statistical tests have when necessary been weighed and compared over several age classes

Type of education

The subjects were divided into three groups with different educational background i e subjects who had passed

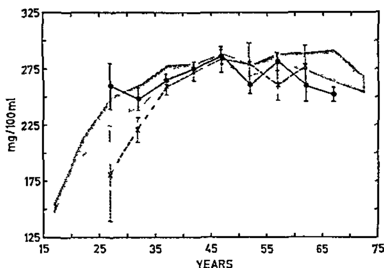


Fig 13 Serum cholesterol concentration (mean value ± standard error of the mean) and age in healthy men (group 10) with different educational background Shaded area elementary school ●—● high school x - - x academic degree

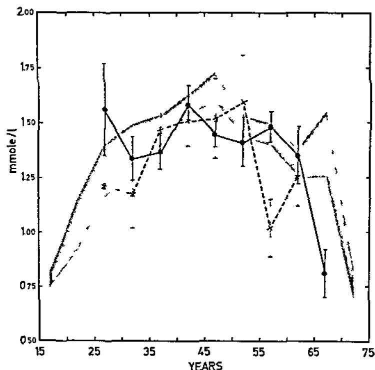
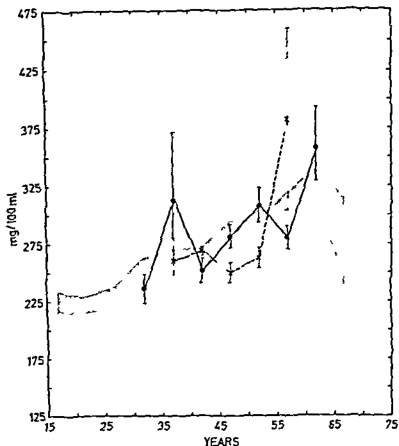


Fig 14 Serum triglyceride concentration (mean value ± standard error of the mean) and age in healthy men (group 10) with different educational background Shaded area elementary school ●—● high school x - - x academic degree



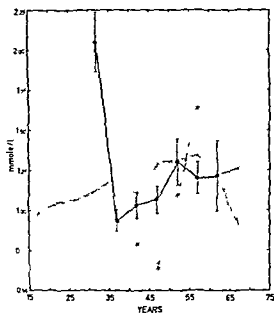


Fig 15 Serum cholesterol concentration (mean value ± standard error of the mean) and age in healthy women *(group 10)* with different educational background Shaded area elementary school ●—● high school x x academic degree

Fig 16 Serum triglyceride concentration (mean value ± standard error of the mean) and age in healthy women (group 10) with different educational background Shaded area elementary school ●—● high school x x academic degree

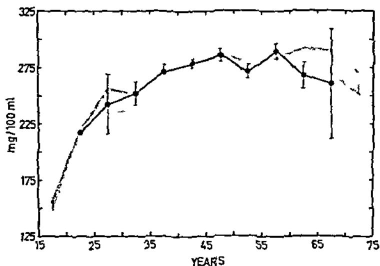


Fig 17 Serum cholesterol concentration (mean value ± standard error of the mean) and age in healthy men (group 10) in subordinate and in supervisory positions Shaded area subordinate positions ●–● supervisory positions

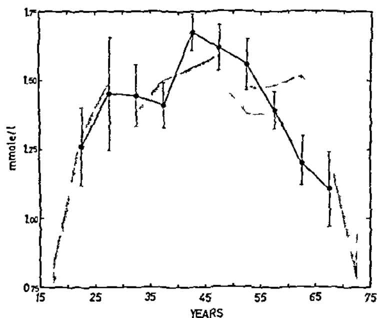


Fig 18 Serum triglyceride concentration (mean value ± standard error of the mean) and age in healthy men (group 10) in subordinate and in supervisory positions Shaded area subordinate positions ●–● supervisory positions

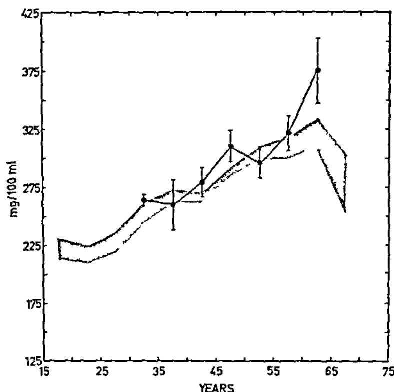


Fig 19 Serum cholesterol concentration (mean value ± standard error of the mean) and age in healthy women (group 10) in subordinate and in supervisory positions Shaded area subordinate positions ●—● supervisory positions

elementary school, subjects who had passed high school ('studentexamen') and subjects who had obtained a university degree The values for blood lipids in these three groups are given in Figures 13 to 16 and in Table A 53 Values for blood pressures and weight height index are given in Tables A 54 and A 55

There were no statistically significant differences between the serum lipid values in the three groups in the different age classes Both men and women with a high school education tended to have lower weight height index than those with an elementary school education, whereas those with a university degree had approximately the same index as those with an elementary school training

Position at work

The subjects were divided into two groups comprising persons in supervisory and in subordinate positions respectively The blood lipid values in these two groups are given for the different age classes in Figures 17 to 20 and in Table A 56 Values for blood pressures and weight height index are given in Tables

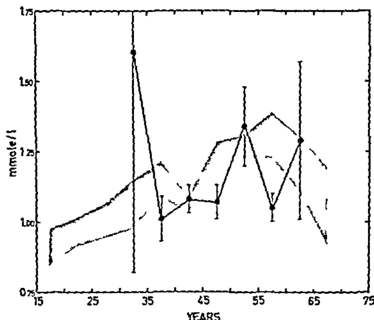


Fig 20 Serum triglyceride concentration (mean value ± standard error of the mean) and age in healthy women (group 10) in subordinate and in supervisory positions Shaded area subordinate positions ●—● supervisory positions

A 57 and A 58

Cholesterol values for men were not significantly different between the two groups There were few women in supervisory positions and although the cholesterol concentration was somewhat higher in this group the difference to the subordinate group was not statistically significant.

The triglyceride level was almost consistently higher for men in supervisory positions than for men in subordinate positions up to the age of 55 whereas the reverse was true for the higher age classes The difference up to the age of 55 was of borderline statistical significance ($P < 0.05$) In women the triglycerides did not differ between the two occupational groups

There was no important difference between the groups with regard to systolic blood pressure. The diastolic pressure was higher in most age classes in men in supervisory positions and the difference was statistically significant The two female groups did not differ significantly in their blood pressures

The weight height index was higher for men in supervisory positions up to 55 years and the difference was of borderline significance ($P < 0.05$) At higher ages men in supervisory positions had slightly lower values for weight height index but the difference from men in subordinate positions was not statistically significant. In women similar variations as in men were observed but the differences between the groups

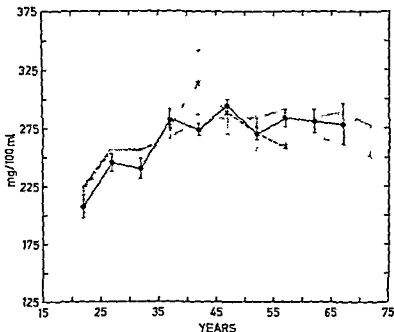


Fig 21 Serum cholesterol concentration (mean value ± standard error of the mean) and age in healthy men (group 10) with different degrees of physical activity at work Shaded area no physical activity ●—● moderate physical activity x x hard physical activity

were not statistically significant

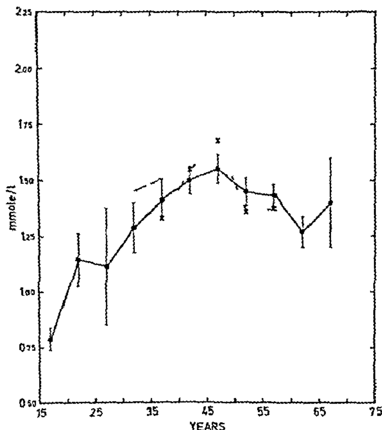
Degree of physical activity during working hours

The subjects were divided into three groups of subjects who reported different degrees of physical activity during working hours i e no physical activity moderate and hard physical activity The blood lipid values are given in Figures 21 to 24 and in Table A 59 for all age classes Values for blood pressures and weight height index are given in Tables A 60 and A 61

There were no consistent differences in the cholesterol values between the three groups for either men or women

The triglyceride values tended to be slightly higher in women with moderate physical activity than in women with no physical activity up to 40 years of age Otherwise the triglyceride values did not differ between the groups with different degrees of physical activity at work

Men reporting no physical activity at work had significantly lower values for systolic blood pressure than men reporting moderate activity Men reporting hard physical activity had still higher values than men with moderate physical activity but since the number of such men was low this difference was not significant Women showed the same relation as men between systolic blood



F g 22 Serum g ce de concen ra on mean al e s anda d *error of he mean and age n* t ea hv men group 10 h d fferen degrees of ph s cal ac y a wo k Shaded a ea no ph s cal acu) ● ● modera e phys cal ac y x x hard physical acti ty

pressure and pl s cal act ty but the d fferences were smaller and not tat st call *s gn f cant* Anal s s of the values for he d astol c blood pressure gave s m lar results as described for the s stol c *pressure*

The e ght he ght ndex was sl ghtly lo er n men th no ph sical act ty than for those th moderate act ity Th s as espec ally true above the age of 50 years P<0 01) Men report ng hard ph sical act ity had h gh values for e ght he ght index but the d f ference from the group th moderate pl s cal activ ty as not *sign f cant* In women the ndex as generall h gher n the nact e group n the younger ages and lo er at older ages

Degree of phys cal act ity during t me off work

The subjects were d ided nto three groups reporting no modera e or hard

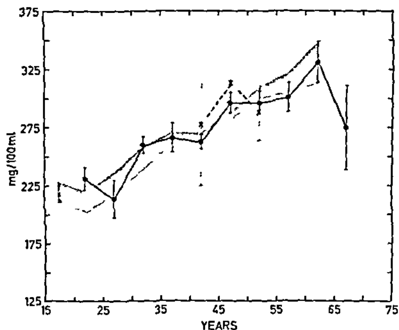


Fig 23 Serum cholesterol concentration (mean value ± standard error of the mean) and age in healthy women (group 10) with different degrees of physical activity at work Shaded area no physical activity ●—● moderate physical activity x - x hard physical activity

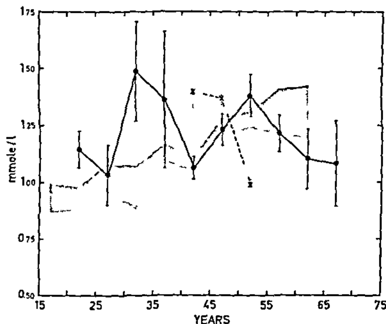


Fig 24 Serum triglyceride concentration (mean value ± standard error of the mean) and age in healthy women (group 10) with different degrees of physical activity at work Shaded area no physical activity ●—● moderate physical activity x x hard physical activity

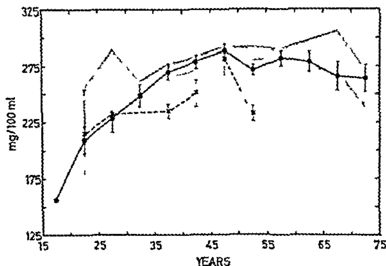


Fig 25 Serum cholesterol concentration (mean value ± standard error of the mean) and age in healthy men (group 10) with different degrees of physical activity during off work hours Shaded area no physical activity ●—● moderate physical activity x x hard physical activity

physical activity during time off work The blood lipid values are given in Figures 25 to 28 and in Table A 62 for the different age classes Values for blood pressures and weight height index are given in Tables A 63 and A 64

The cholesterol values in men reporting no or moderate physical activity did not differ significantly On the other hand in all age classes men reporting hard physical activity had a lower cholesterol level—on the average 30 mg/100 ml—than men reporting no physical activity There were no major differences in the concentration of cholesterol between active and inactive women although the values tended to be lower for active women between 30 to 40 years

In men reporting moderate physical activity during time off work the concentration of serum triglycerides in all age classes was generally about 0 15 mmole per l lower than in those who reported no physical activity ($P<0\ 001$) In those reporting hard physical activity it was about 0 40 mmole per l lower than in the inactive group (Fig 26)

The differences between men reporting moderate and hard activity with respect to both cholesterol and triglyceride values were of borderline statistical significance ($P<0\ 05$) Women showed no difference in triglyceride values in relation to reported physical activity

Men and women reporting moderate physical activity did not differ in weight height index from those reporting none

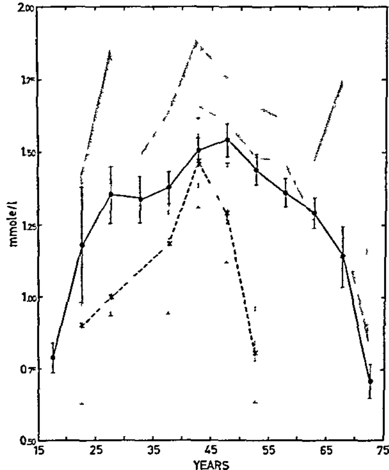


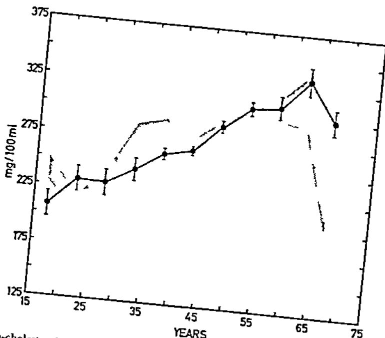
Fig 26 Serum triglyceride concentration (mean value ± standard error of the mean) and age in healthy men (group 10) with different degrees of physical activity during off work hours Shaded area no physical activity ●—● moderate physical activity x x hard physical activity

However men with hard physical activity had a lower index.

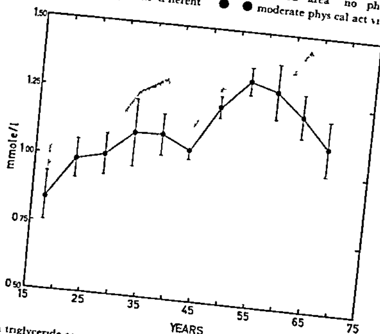
Smoking

The subjects were divided into three groups according to their smoking habits i.e. non smokers moderate smokers (less than 20 cigarettes or their equivalent per day) and heavy smokers (more than 20 cigarettes or their equivalent per day) The blood lipid values for these groups are given in Figures 29 to 32 and in Table A 65 for the different age classes Values for the blood pressure and for the weight height index are given in Tables A 66 and A 67

There was no difference in cholesterol values between moderate and heavy male smokers and these subgroups have been



F g 27 Serum-cholesterol concentration (mean value ± standard error of the mean) and age n healthy women (group 10) w th d fferent degrees of phys cal act ty during off work hours Shaded area no phys cal act ity ● ● moderate phys cal act vity



F g 28 Serum triglyceride concentrat on (mean alue standard error of the mean) and age n heal hy omen (group 10 w th d fferent degrees of phys cal act ty during off work hours Shaded area no phys cal act ity ●—● moderate phys cal act ty

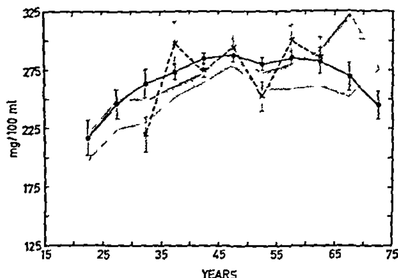


Fig 29 Serum-cholesterol concentration (mean value ± standard error of the mean) and age in healthy men (group 10) with different smoking habits Shaded area no smoking, ●—● moderate smoking i e less than 20 cigarettes or their equivalent per day x - x heavy smoking i e more than 20 cigarettes or their equivalent per day

combined into one group smokers Male smokers had significantly higher cholesterol values than non smokers Men, previously moderate smokers, who had stopped smoking two or more years before, did not differ significantly with regard to cholesterol levels from either moderate smokers or non smokers There was, however a tendency for these subjects to have lower cholesterol values than smokers but the group is too small to permit definite conclusions The same applies to previously heavy smokers

Women with different smoking habits did not differ with regard to the con centration of cholesterol in serum.

The levels of triglycerides were signif icantly higher for both heavy and mod erate male smokers than for non smokers Heavily smoking men usually had higher levels than moderate smokers, but the difference was not statistically significant As in the case of cholesterol those who had stopped smoking did not differ significantly from non smokers Female smokers also had significantly higher triglyceride values than did non-smokers

The systolic and diastolic blood pres sures showed the same relationship to smoking habits Male non smokers and moderate smokers had similar values, whereas heavy smokers had significantly higher blood pressures Men previously moderate smokers who had refrained from smoking for more than two years showed a tendency towards higher blood pressures than moderate smokers The picture was different for women, where smokers tended to have lower values

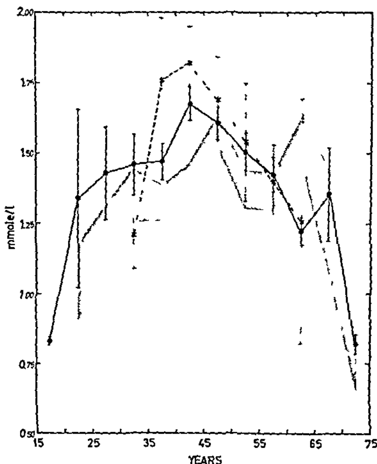


Fig 30 Serum triglyceride concentration (mean value ± standard error of the mean) and age in healthy men (group 10) with different smoking habits Shaded area no smoking ●—● moderate smoking i e less than 20 cigarettes or their equivalent per day x x heavy smoking i e more than 20 cigarettes or their equivalent per day

for blood pressure than non smokers The difference is however only of border line statistical significance

Non smoking and smoking men had similar values for weight height index Interestingly, previously moderate smokers had significantly higher values than moderately smoking men Moderately smoking women had an almost significantly lower weight height index than non smoking women

Correlations between the plasma lipids blood pressures and weight height index

Partial correlation coefficients keeping

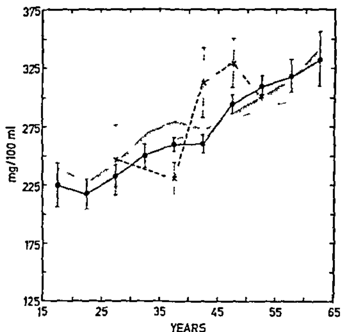


Fig 31 Serum cholesterol concentration (mean value ± standard error of the mean) and age in healthy women (group 10) with different smoking habits Shaded area no smoking ●—● moderate smoking i e less than 20 cigarettes or their equivalent per day x x heavy smoking i e more than 20 cigarettes or their equivalent per day

age constant between the two lipid values and also for different lipid values and other parameters in group 10 are given in Tables A 68 to A 72

TABLE VIII Partial correlation coefficient (age constant) for concentration of cholesterol on concentration of triglycerides in group 10 for men and women

	N	r
Men	1266	0 358***
Women	949	0 209***

Symbols see Table IX

Table VIII shows that there was a significant and positive correlation between the values for cholesterol and triglycerides This correlation was higher for men The correlation coefficients in the different age classes are given in Table A 68 which shows that there was no decided age trend for the inter lipid correlation

There was only very slight correlation between blood pressures and lipid values in the entire group (Table IX) for both men and women Tables A 69 and A 70 give the correlation coefficients in the different age classes

The correlations between the plasma lipid values and the weight height index

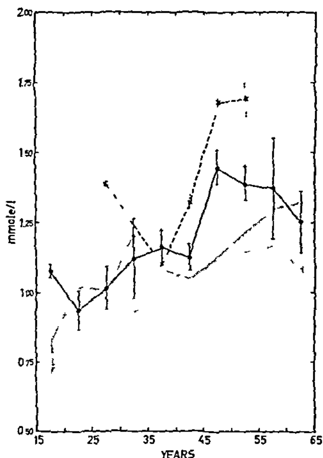


Fig 32 Serum triglyceride concentration (mean value ± standard error of the mean) and age in healthy women (group 10) with different smoking habits Shaded area no smoking ●—● moderate smoking i e less than 20 cigarettes or their equivalent per day x x heavy smoking i e more than 20 cigarettes or their equivalent per day

are given in Table X for all ages and in Table A 71 for the different age classes For men there was a significant correlation between both cholesterol and triglycerides and the weight height index most marked for the triglycerides For women there was a correlation only between plasma triglycerides and this index Furthermore this correlation was much lower for women than for men

The correlations between the weight-height index and blood pressures were around 0 21 for men and 0 25 for women (Table XI and Table A 72)

TABLE IX. Partial correlation coefficients (age constant) for the correlation between systolic and diastolic blood pressures on respectively concentrations of cholesterol and triglycerides and on log concentration of triglycerides in group 10 for men and women

	N	*r* systolic blood pressure and			*r* diastolic blood pressure and		
		cholesterol	triglycerides	log triglycerides	cholesterol	triglycerides	log triglycerides
Men	1266	0 041	0 050	0 065*	0 098**	0 045	0 063*
Women	949	0 068*	0 054	0 070*	0 085**	0 056	0 078*

*, ** and *** indicate that the statistical significance of the correlation coefficient is P < 0 05 < 0 01 and < 0 001

TABLE X Partial correlation coefficients (age constant) for cholesterol and triglyceride concentrations on the weight height index in group 10 for men and women

	N	weight/height index and	
		cholesterol	triglycerides
Men	1266	0 130***	0 231***
Women	949	0 038	0 085*

Symbols see Table IX

TABLE XI Partial correlation coefficients (age constant) for the weight height index on systolic and diastolic blood pressures in group 10 for men and women

	N	weight/height index and	
		systolic pressure	diastolic pressure
Men	1266	0 211***	0 209***
Women	949	0 260***	0 252***

Symbols see Table IX

Weekday and seasonal variation

In order to establish if there was any variation in lipid values over the year or during the week the mean values were listed for each weekday and month

The cholesterol levels did not differ significantly in either men or women on the different weekdays from Monday to Friday (Fig 33, Tables A 73 and A 74) The triglyceride values also remained constant in both men and women throughout the week (Fig 34 Tables A 75 and A 76)

The values for cholesterol followed the same pattern in men and women during the year (Tables A 77 and A 78) Figure 35 shows that the level was fairly constant from January to May but then declined and reached a minimum level in July The concentration of cholesterol increased from July to September—October and then remained at a rather constant level The maximal differences in cholesterol level during the year were 38 and 29 mg per 100 ml for men and women respectively

The variation of the serum triglycerides over the year is given in Figure 36 and Tables A 79 and A 80 The most striking feature was the low level

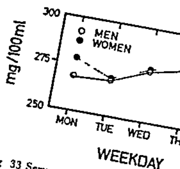


Fig 33 Serum cholesterol concentratio… value) in healthy men and women (gr… on different weekdays

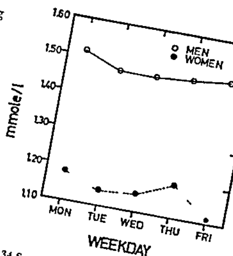


Fig 34 Serum triglyceride concentration (mean value) in healthy men and women (group 10) on different weekdays

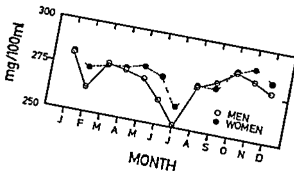


Fig 35 Serum-cholesterol concentration (mean value) in healthy men and women (group 10) in different months during the year

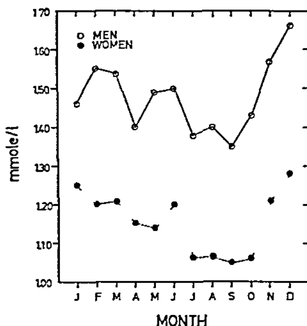


Fig 36 Serum triglyceride concentration (mean value) in healthy men and women (group 10) in different months during the year

seen in July, August and September—for women also in October—which was followed by a pronounced increase to a maximum level in December

DISCUSSION

Introduction

The present study like many others in the same field is not of an experimental nature and it is clear that many factors are involved in the selection of subjects for study Thus there is a predominance of white collar workers due to the type of companies conducting the health control program Although it is known that of those offered the yearly examination the majority avail themselves of this opportunity we do not know if there is any difference between those who were not examined—or those who did not wish to participate in this study—and those who took part This calls for caution in the statistical analysis and in the evaluation of the results Statistically significant differences are to be expected in a population of this size but their biological meaning will depend on the magnitude of the observed differences or correlations between variables They must also be viewed against a background of other studies and against our general knowledge of lipid metabolism

The connection between the level of blood cholesterol and coronary heart disease (97 59 4) has led to a large number of population studies during the last fifteen years in order to define the "normal" level of blood cholesterol and to elucidate the factors that regulate this level Among the many factors that have been considered in these studies of apparently healthy subjects are sex and age the ethnic and geographical origin of the subjects heredity type of society in which the individual lives—primitive *versus* urbanized society—the diet physical activity smoking habits etc It has not been possible to isolate the influence of any single factor on the serum cholesterol level since this would require very large groups of subjects As one example there is an obvious relation between the total caloric intake energy expenditure and body weight which are all factors that have been postulated to influence the level of serum cholesterol Direct proof of a causal relationship between the serum cholesterol concentration and any of the above mentioned factors is therefore still lacking and it will probably not be found until the underlying biochemical mechanisms which regulate the level of blood lipids have been established

The recent demonstration that the concentration of serum triglycerides may also be elevated in coronary heart disease (6 22 11 50) has stimulated inter-

est in this fraction of the blood lipids However, relatively few population *studies concerning levels of triglycerides* have been reported, and the influence of environmental and other factors on this lipid fraction has been studied only to a limited extent in small groups

Frequency distribution of lipid values

The frequency distribution of values for serum lipids has been tested for normal distribution in some previous studies Thus Josephson and Dahlberg (54) found no significant skewness in the frequency distribution of cholesterol values nor did Page and co-workers (90) in a small group of 284 subjects In the large Minnesota sample Keys and co-workers found the cholesterol values to be normally distributed (65) A skewness to the right in the frequency distribution of serum cholesterol values was noted, however, by Lewis and co-workers (72) who also observed an even more marked skewness in the distribution of the lipoprotein concentration values Cornfield and co-workers (27) showed that the logarithms of the cholesterol concentration values were normally distributed A skewed distribution of triglyceride concentrations has been observed by Page and co-workers (90) and by Carlson (21) particularly in the younger age class The authors have discussed the use of logarithmically transformed concentrations as suggested by Gaddum (40), which were used in the statistical calculations in Carlson s work and subsequently also by others (28 36) In the present study the distributions were tested for skewness in all age classes and were found to be skewed to the right for *cholesterol in several age classes and for* triglycerides in almost all age classes The skewness was considerably more pronounced for the triglyceride values than for the cholesterol values It could be considerably reduced for the triglycerides by using the transformation to logarithmic values which were therefore *also used in the correlation and regres*sion analyses The conclusions were, however essentially the same whether the calculations were carried out with the concentration values or with their logarithms The large biological dispersion in the values for the serum lipid concentrations is reflected in the large standard deviations obtained in all age classes In the case of cholesterol standard deviations of around 30 to 50 mg per 100 ml have been obtained in previous studies (65 72 3), as was also the case in this study Schaefer (96) found a noticeable skewness in the frequency distribution of triglyceride concentrations and also a second peak, at 180 to 200 mg per 100 ml, in the frequency distribution of triglyceride values of men aged 33 to 79 compared to a mean concentration of 60 to 80 mg per 100 ml in the main peak (For comparison of concentration values, 1 mmole per l with the method used in this study is equivalent to about 85 mg per 100 ml) The second population comprised 12 per cent of the total number Antonis and Bersohn (11) have also interpreted their results as showing the presence of a second population of triglyceride values which possibly contains potential cases of heart disease In the present study the standard deviations

were about the same in most age classes The frequency distribution was normalized in almost all age classes after the logarithmic transformation, and there is thus no evidence for the presence of two populations in our material

Age, sex and lipid levels

In the western urbanized society the blood cholesterol concentration in men rises from the age of 20 years to about 50 to 60 years remains on a plateau for about a decade and then declines at higher ages In women the concentration of blood cholesterol is lower than in men during the younger years but continues to rise after the age of 50 years Thus results in a higher concentration of serum cholesterol in women than in men of the older age classes In many primitive societies as well as in the poorer groups in some western societies the blood cholesterol concentration is lower than in the wealthier groups of an urbanized western society The concentration of serum cholesterol in these groups may increase between 20 and 30 years but then usually remains at a constant level However the question of whether the serum cholesterol concentration increases with age has been the subject of some controversy On the basis of early studies in American populations it was concluded that no such increase with age occurred (90 103 77) and in 1953 Oliver and Boyd (89) found no increase with age in either sex among a group of convalescent in patients In 1950 Keys (65) reported on the concentration of serum cholesterol in 1492 men and 564 women from a wealthy population in Minnesota who were healthy as judged by physical examination In men a steady increase in the concentration of serum cholesterol of 2 29 mg per 100 ml per year occurred from 17 to 45 years whereafter the concentration remained on a plateau of about 250 mg per 100 ml until about 60 years of age This was followed by a decline with 2 9 mg per 100 ml per year There was no difference between men and women of the ages 17 to 30 years Adlersberg and co-workers (3) determined the concentration of serum cholesterol in 1200 subjects aged 2 to 77 years in the lower income bracket from Staten Island N Y USA In this study the serum cholesterol concentration increased in men by 3 6 mg per 100 ml per year from 20 to 33 years and then remained constant at a level of about 240 mg per 100 ml In women there was a steady increase of about 3 mg per year from 20 years to 60 years of age at which time a maximal concentration of 286 mg per 100 ml was reached In the last mentioned study the serum cholesterol concentration in men was higher than in women of the ages 28 to 42 years Subsequent studies on American populations have in general confirmed these findings For instance the results of a large cooperative study carried out to evaluate the possible significance of the atherogenic index proposed by Gofman (41) have been reported by Lewis and co-workers (72) The total number of subjects was about 10 000 men and 3000 women from different parts of the USA In men a plateau level of about 250 mg per 100 ml was reached at 40 to 45 years of age whereas in women the concentration continued to rise to about

270 mg per 100 ml at the age of 60 years Between 30 and 45 years the concentration of serum cholesterol was about 20 mg per 100 ml lower in women than in men

Keys (62) has extended his population studies to clinically healthy men in Naples Italy From 20 to 30 years the serum cholesterol concentration increased by about 3 mg per 100 ml per year, then no further increase occurred and the concentration at ages above 30 years remained about 30 mg per 100 ml lower in the Neapolitan men than in the Minnesotans (65) The same pattern was observed in a study of serum cholesterol concentration in a group of poor people in Spain (66) in whom the cholesterol concentration was even lower than in the Neapolitan or Minnesotan material The difference between the American and the Mediterranean populations was apparently not due to race differences since professional people in Madrid, Spain were found to have a blood cholesterol level of the American type Furthermore, Italians residing in Boston (83, 53 76) have the same cholesterol pattern as Americans This is also the case for Japanese living in California although Japanese living in Japan have lower cholesterol values than Americans and show an insignificant increase in serum cholesterol concentration with age (64) Other studies in poor or primitive societies have demonstrated the characteristic absence of a correlation between serum cholesterol concentration and age in these types of populations (82 79 98, 78 111, 100, 99 80) The large variation that may be encountered between different populations is evident from the fact that at ages 40 to 49 years farmers in Koga, Japan, have a cholesterol level almost 100 mg per 100 ml below that of Americans in Minnesota (64) Keys has postulated that the decisive factor in the environment which regulates the cholesterol level is the amount of fat calories in the diet, since he could demonstrate a linear correlation between the level of blood cholesterol and calories derived from fat in a number of populations of different origin (64) Later work has clearly demonstrated the importance not only of total fat intake but also of the relationship between saturated and unsaturated fat in the diet (68, 18, 5)

The relationship between serum cholesterol concentration and age which was found in the present study resembles that found in most studies of urbanized western societies In contrast to the findings in some American groups (72, 3), there was no significant difference between men and women in the younger ages except for the age class 15 to 19 years It is of interest to compare results from previous studies of Scandinavian populations with the present study In 1952 Josephson and Dahlberg (54) determined the serum cholesterol concentration in 527 healthy men and 193 healthy women in Stockholm The concentration in both sexes was around 220 mg per 100 ml between 16 and 60 years but the level declined at higher ages Lindholm in 1956 (73) determined the serum cholesterol concentration in 102 healthy men and 93 healthy women from the southern Swedish province Skane In men there was a maximal concentration around 180 mg per

100 ml at 40 to 49 years *In women* there occurred a rise of 1 1 mg per 100 ml per year until the age of 70 to 79 years *when the serum cholesterol con*centration had reached 223 mg per 100 ml Carlson in 1960 (21) reported on the *serum cholesterol concentration* in 93 clinically healthy males aged 26 to 55 years In this group there was a statistically significant rise in the serum cholesterol concentration of 1 5 mg per 100 ml per year over the entire age span At 46 to 55 years the cholesterol level was about 270 mg per 100 ml a figure which is in good agreement with that obtained in this study Svanborg and Svennerholm (104) have determined the serum cholesterol concentration in 62 men and 29 women aged 16 to 35 years from Gothenburg Sweden The mean values were 191 5 mg per 100 ml in men and 185 4 mg per 100 ml in women In a recent study in Oslo Norway Berge and Nicolaysen (14) found a mean value of 270 mg per 100 ml in 6967 healthy men aged *40 to 59 years and of various* occupations An unusually large annual increase in the serum *cholesterol* con*centration of 4 6 mg per* 100 ml per year was found in 487 men aged 20 to 39 years employed in the Oslo telegraph service and a similar increase was also noted in a group of blood donors (84) in which however the cholesterol concentration was 20 to 40 mg lower in all ages than in the other two groups studied i e men in various occupations and men employed in the telegraph service The variation in serum cholesterol concentration observed in the blood donors in Oslo was similar *to* that found *in blood donors in Copenhagen* (74) Other reports on the *level of serum* cholesterol in Denmark have been published by Kornerup (71) and by Lund and coworkers (75) *In Finland the serum* cholesterol concentration has been determined in several population groups (85, 63 58 93 92 61)

A relationship between the concentration of serum triglycerides and age may be inferred from early studies employing less specific analytical methods and from the positive correlation between the concentration of low density lipoproteins and age (72) In 1960 Carlson used a specific analytical method to examine this question in a selected material of healthy men (21) The concentration of serum triglycerides was 0 96 mmole per l at 26 to 30 years and it increased significantly to the age of 40 when a level of 1 2 to 1 4 mmole per l was reached. This level remained constant to 73 years The present study was done in the same city with the same analytical technique The level of triglycerides at the ages 35 to 55 years *was about 0 3 mmole per l* above that found by Carlson in 1960 The subjects included in that study were all examined by the same physician and ECG s were also recorded both during and after exercise It is possible that this may have contributed to the *lower levels* found by Carlson in his normal group Schaefer (96) has determined serum triglyceride concentration in 433 healthy adults from New York City without clinical evidence of ischemic heart disease In women the median serum triglyceride value rose from 54 mg per 100 ml at the age of 30 years to 80 mg per 100 ml at 64 years of age This was followed by a *moderate decline* at higher ages In

men there was a steep rise in the concentration from 70 to 80 mg per 100 ml at age 41 to a peak value of 106 mg per 100 ml at age 47, followed by a return to concentrations around 80 mg per 100 ml at age 60 and thereafter. Svanborg and Svennerholm (104) have reported 83.5 mg per 100 ml in men and 88 mg per 100 ml in women aged 16 to 35 years from a study of 91 subjects in Gothenburg, Sweden. In the same city Cramer (28) found mean values of 0.65 and 0.61 mmole per l for healthy men and women in the ages 20 to 40 years which increased to 0.79 and 0.96 mmole per l in the age class 55 to 65 years. Antonis and Bersohn (11) have reported similar figures for European and Bantu males under 40 years of age, whereas the concentration of triglycerides in women was about 20 mg per 100 ml lower. In the European males a definite age trend was found, but the triglyceride concentration remained constant in the Bantu males from 20 to 60 years. An increase in the triglyceride concentration with age has also been reported by Albrink and co-workers (6, 9), by Furman and collaborators (39) and in women by Feldman and co-workers (36), whereas Hayes and Neill (50) did not find any relation between age and serum triglycerides in men or women, either normal or with coronary heart disease. The age trend for triglycerides found in this study is thus in general agreement with results obtained in other studies of similar populations. The significant decrease in the concentration of serum triglycerides in men above 50 years of age which was found in this study has not been observed in other population studies.

Correlation between triglycerides and cholesterol

The fact that only a low grade partial correlation existed between the concentration of cholesterol and triglycerides is not unexpected as they are mainly contained in different lipoprotein classes. The concentrations of the cholesterol rich low density lipoproteins and the triglyceride rich very low density lipoproteins may vary independently as is well known from experimental and clinical studies. Furthermore the Gofman group found no correlation between the concentration of low density (S_f 0—12) and very low density lipoproteins (41). A low grade correlation between cholesterol and triglycerides has been found in both normal men (21) and in men with coronary heart disease (22).

The low grade correlation between these two serum lipids stresses the importance of a study where both are determined.

Environmental factors and plasma lipids

In the present study the material was classified by a method of successive exclusions. Thus, a group of subjects suffering from various diseases (group 1) and finally a healthy group (group 10) were obtained. The remaining eight subgroups contained subjects with heredity for or signs of disease of particular interest to the follow up study of the ma

terial Except for the group of obese subjects none of these groups differed significantly from the healthy group with regard to the level of serum cholesterol or serum triglycerides In group 10 comprising presumably healthy subjects, a number of factors were studied, some of which have been suggested to be of importance for the development of coronary heart disease (cf 97, 59)

Only one factor at a time has been used in the classification and the subgroups are not to be regarded as homogenous populations In many cases the classification has been based on the subjective opinion of the subjects (i e no moderate or hard physical activity) and the influence of variables other than that which is the basis for the classification into the different groups cannot be excluded The findings should mainly be regarded as a description of the different groups and the statistical analyses as indications of relations between variables which may not necessarily be causally related to each other

Position at work

Men in supervisory positions up to age about 50 years tended to have slightly raised levels of cholesterol and triglycerides No directly comparable studies are available Lewis et al (72) found no major difference in the serum cholesterol values of American males age 40 to 60 years of different social classes and occupations Of the variety of physical psychological and environmental factors that may differ between men in supervisory and subordinate positions it may be relevant that emotional stress can increase the concentration of cholesterol triglycerides and very low density lipoproteins (37, 109 113 44, 45, 24) It is also of interest to note that in those age classes where the triglyceride levels were elevated the weight height index was also increased for men in supervisory positions (see section on body weight)

Physical activity

In the present study the subjects were asked to give a subjective estimate of their degree of physical activity during working hours as well as during time off work Whereas no significant difference was found between the lipid values of subjects reporting various degrees of physical activity during working hours these values tended to be lower in men reporting some physical activity during time off work than for those reporting no physical activity off work This was most marked in the case of triglycerides which were as much as 0 40 mmole per l lower in the group with hard physical activity than in the physically inactive group A highly significant difference was also found in the concentration of serum cholesterol which was about 30 mg per 100 ml lower in men reporting hard physical activity than in the other two groups

In the previously mentioned study by Keys of a Neapolitan population (62) the material was divided into two groups i e firemen with hard physical training and vigili annonari with light physical work It was concluded that differences in physical activity did not influence the serum cholesterol level as has also been the conclusion of other studies where this question has been examined (10o

81, 43) On the other hand, Miller and co-workers (83) who divided their material of Italian men in Boston into three groups comprising subjects with less than usual, usual and more than usual physical activity found the highest cholesterol concentration in the sedentary group followed by those with more than usual and usual physical activity In a 4 year follow up study of the same material, however, no relation existed between physical activity and serum cholesterol concentration (53) Keys, Karvonen and Fidanza (63) in 1958 studied groups from east and west Finland and classified the material according to the level of occupational physical activity In men with light and heavy physical work the concentration at ages 30 to 39 was 276 and 263 mg per 100 ml respectively in the eastern parts of the country, whereas the corresponding figures for the western part were 212 and 223 mg per 100 ml Mann and co workers concluded from their studies (78, 79) that physical activity may influence the serum lipoprotein and cholesterol concentration An association between strenuous physical activity and low serum cholesterol levels has been found by Golding (43) and by Rochelle (95) Also regular endurance exercise has been found to reduce the level of triglycerides (52) It is of interest that physical exercise of some hours duration has also been found to reduce the postprandial rise in serum triglycerides (26, 86 19) as well as the level of endogenous plasma triglycerides (25) It has been realized in most studies of the relation between physical activity and blood lipids that differences in physical activity between different groups are often associated with differences in dietary habits, body weight etc Gsell and Mayer (46) have reported interesting results from a comparison of the serum cholesterol levels in two populations, one living in a Swiss mountain village and one in Basel It was found that although the village people had a higher caloric intake and consumed relatively more animal fat with saturated fatty acids than the people in Basel, their cholesterol concentration was significantly lower This was ascribed to their very hard physical activity of chasing goats in the Alps To exclude other environmental factors Hernberg (51) studied a group of 1012 leading businessmen in Finland and correlated the capacity for physical work, measured as maximum oxygen uptake, with serum cholesterol concentration A significant negative correlation was found in the ages 40 to 49 years, and a negative correlation of borderline significance in the ages 30 to 39 years was also observed

It was also found in the present study that weight height index and systolic and diastolic blood pressure were lower in men reporting physical activity off work, whereas these parameters were increased in men as well as in women reporting moderate and hard physical activity during working hours The fact that these parameters change in the opposite direction for those reporting increased physical activity on and off work makes it clear that two different subgroups of the population have indeed been segregated The differences in lipid levels between subjects reporting different degrees of psysical activity also tend to be reversed depending on whether the phys-

ical activity occurs at work or not, and these differences cannot at the present state of our knowledge be ascribed to the physical activity as such, but rather as characteristic of two groups of subjects which presumably in many respects show considerable differences in their habits of life

Several recent epidemiological studies have suggested that physical activity may be a positive factor in the prevention of coronary heart disease although this has been doubted by others Our data clearly indicate the importance of distinguishing between physical activity on and off work

Smoking

An association between smoking and coronary heart disease has been noted in several epidemiological studies (47 31 32, 33) and the possible relationship between smoking habits blood pressure, and serum lipids has consequently attracted considerable interest Many groups have reported elevated blood cholesterol values in male smokers particularly in the younger age classes (50 83 53 61 106 17 107 15, 42) No relation between smoking and cholesterol levels was found however, in a study of 314 young men in Finland (69), in a study of Finnish businessmen (51) or in a study of old men in England (2) In our study male smokers were found to have a significantly higher serum cholesterol than non smokers but no difference was noted between heavy and moderate smokers The difference between smokers and non smokers was most marked in the younger and middle age classes Similarly younger and middle aged smoking men had significantly higher triglyceride levels than non smokers The cholesterol concentration in smoking women was elevated only in two age classes but triglyceride concentration was high in most age classes A relation between smoking habits and concentration of serum triglyceride has not been reported previously, but Gofman has reported an elevation of the triglyceride rich class of very low density lipoproteins in young men smoking more than 20 cigarettes a day (42) In short term experiments it has been found that smoking two cigarettes has no effect on the lipoprotein (91) or triglyceride level (60) but it raises free fatty acid levels (60) Konttinen and Rajasalmi (70) reported a smaller postprandial rise in triglycerides among those smoking several cigarettes after a meal than in the non smoking control group

It has been stressed in previous studies that the possible relationship between smoking and coronary heart disease is difficult to evaluate since smoking may also affect the degree of obesity and blood pressure (61) which may in turn be related to any observed differences in the frequency of coronary heart disease In the present study weak correlations between serum lipids and blood pressure serum lipids and weight height index and weight height index and blood pressure were noted Similar weak associations between these factors have also been noted in other studies of large populations

Relative body weight

It has been much discussed whether a

correlation exists between the serum lipids and some relative measurement of obesity Keys expressed the opinion that no such correlation existed for serum cholesterol on the basis of the results from population studies in the USA, Italy and Spain, and this finding was confirmed in a 15 year follow-up of the Minnesotan material The same conclusion has been reached by several others who have used various methods to estimate body fatness, ranging from body weight to different somatological indices Lindholm (73), who used the same weight height index as the present study, found no correlation between this index and the serum cholesterol concentration

In the present study there was no significant difference between the serum cholesterol concentration in men or women referred to the obese group and the concentration found in healthy subjects In the healthy group a weak, but significant age independent correlation was found between the cholesterol level in men and the weight height index, whereas no correlation existed in women Lewis and co workers (72) found a significant—although very low—correlation beween serum cholesterol concentration and weight for both men and women in the ages 30 to 49 years and a significant but very small (1 to 4 per cent) elevation of serum cholesterol in an over weight subgroup of their material Miller and co-workers in 1958 (83) stated that the influence of body weight on cholesterol level is considerable although this is hardly consistent with their reported figures e g the cholesterol level was lower in those with a gross increase in relative weight than in those with a moderate increase and at 30 to 39 years even lower than in those with a normal relative weight No clearcut relation to the thickness of subcutaneous skinfolds or to various bone measurements was noted In a follow up study of the same subjects no tendency for cholesterol to increase with weight could be seen (53, 76) Keys in 1954 (62) showed a correlation between relative body weight and serum cholesterol concentration as well as between skinfold thickness and serum cholesterol in Neapolitans of the working class Since no such correlation existed in subjects from Minnesota, he postulated that a correlation between body weight and serum cholesterol may exist in populations in which the serum cholesterol concentration is generally low but not in populations with a generally high serum cholesterol level Apparently only a weak association exists between various measurements of relative obesity and cholesterol, which may only be noticeable in large population studies

As regards triglycerides there is a more significant relation to relative obesity In the present study, males referred to the obese group were found to have concentrations of triglycerides from 0 3 to 0 5 mmole per l higher than healthy males and a significant age independent correlation was found between the weight height index and the triglyceride concentration of healthy men A significant but less pronounced correlation was also noted between these parameters in healthy women, whereas the triglyceride concentration was not different in the groups of obese and non obese women Obese men in Stockholm (20) and in Gothenburg (13) have previously been

found to have normal cholesterol values but elevated plasma triglycerides Waxler and Craig (112) reported elevated con centrations of serum triglycerides in obese women particularly in those termed latent diabetics on the basis of an oral glucose tolerance test Albrink and co-workers (7, 8) have found a cor relation between weight gain in adult life and triglyceride concentration as well as between various measures of body fatness including skinfold thickness and triglyceride concentration In a large population study Lewis and co-workers (72) found low but significant cor relations between the concentrations of the triglyceride rich lipoproteins S_f 12—20 and S_f 20—100 and weight

Weekday and seasonal variation

Any influence of the time factor on the fasting levels of serum lipids is of impor tance in all studies on lipid levels This applies to studies considered independent of time such as those carried out to establish normal values as well as to time dependent studies such as studies on effects of drugs

Sampling of blood on different week days had no apparent influence on the lipid values in either sex On the other hand in both sexes there was a definite influence of the season on the con centration of cholesterol as well as of tri glycerides Both lipids were lowest in the summer and then increased during the autumn

It is of interest, however, that the seasonal variations of the cholesterol and triglyceride levels were not parallel While the cholesterol concentration in creased sharply from July onwards the concentration of triglycerides continued to remain at a low level in August and September and for women also in Oc tober This indicates that different sea sonal factors are involved

A seasonal variation in the cholesterol level has been noted earlier in several studies (63, 92 29 94 55, 108) The most marked seasonal effect was re ported by Keys and co-workers in East Finland (63) where the values for serum cholesterol increased from June to January by about 100 mg per 100 ml This increase is about 3 times the in crease observed in the present material from Stockholm The serum triglycerides have to our knowledge only been studied in young men in relation to season in Antarctica where no seasonal variation was found (12)

SUMMARY

The Stockholm Prospective Study is planned to determine the relative merits of the serum concentration of cholesterol and triglycerides as well as of other potential "risk factors" in healthy persons in predicting the future development of coronary heart disease. The sampling and examination of the subjects included in this study in 1961—1962 is described. The values for the serum lipids and their correlation to various factors as well as values for blood pressure and for weight height relation are given in this report.

In all 6464 subjects, 15 to 74 years, 3624 men and 2840 women, were studied. By a method of successive exclusion of groups of persons with different diagnoses a final, healthy group was arrived at. There were no major differences between the lipid values in the healthy group and in the different groups with various diseases and conditions except for obese men, who had significantly elevated triglyceride levels.

The following major observations were made on the serum lipids in the healthy group.

a) Serum cholesterol values showed a moderate and serum triglyceride values a pronounced skewness in frequency distribution with the mean greater than the median. The skewness for the triglyceride values was reduced by logarithmic transformation.

b) In the age class 15 to 19 years women had higher cholesterol levels than men. At 20 to 24 years the levels were the same in both sexes and increased parallelly up to 50 years and continued to increase in women while it levelled off in men. Women thus had significantly higher cholesterol values than men at 50 to 64 years.

c) The serum triglyceride concentration was the same in men and women at 15 to 19 years. In men it increased to a maximum at 45 years and then decreased significantly. In women it increased to about 55 years. Between 25 and 55 years the male level was about 25 per cent higher than the female.

d) The serum lipid values did not differ between groups with different types of education.

e) Men in supervisory positions at work tended to have slightly higher serum lipid values than men in subordinate positions up to about 50 years. There were less pronounced differences between women in different positions.

f) The serum lipid values did not differ between groups with different degrees of physical activity during working hours.

g) Men reporting hard physical activity during time off work had in all age classes lower values for cholesterol and triglycerides—10 and 25 per cent respectively—than men reporting no such activity Men reporting moderate physical activity off work had slightly lower triglyceride level but the same cholesterol level as inactive men There was no consistent difference in lipid values for women reporting no and moderate physical activity off work

h) Cholesterol and triglyceride values were generally higher in smoking men than in non smoking up to 55 years Smoking women had elevated triglyceride levels in all age classes and a tendency to elevated cholesterol values in the higher age classes in comparison with non smoking women

i) The lipid levels showed no systematic variation with the weekdays in either sex

j) The lipid values varied significantly with the season similarly for men and women Cholesterol values were lowest in June—July increased to September——October and remained constant November to May Triglycerides were lowest in July to September and then increased and reached a maximum level in December

ACKNOWLEDGEMENTS

This study was supported by a grant from Folksam Insurance Company, Stockholm

Professors Sune Bergstrom, Gunnar Biorck and Torgny Sjostrand and Dr Rune Frisk participated in the initial planning of the study Dr Sten Aleman Foretagens Halsokontroll and Dr Rune Frisk have assisted in aspects on the organization of the medical examinations of the study

Mr Carl Axel Svedberg Folksam Insurance Company, made the computer program and has been responsible for the data processing

REFERENCES

1 Abel L L Levy B B Brodie B B & Kendall F E Simplified method for the estimation of total cholesterol in serum and demonstration of its specificity J biol Chem *195* 357 1952

2 Acheson R M & Jessop W J E Tobacco smoking and serum lipids in old men Brit med J *2* 1108 1961

3 Adlersberg D Schaefer L E Steinberg A G & Wang C I Age sex serum lipids and coronary atherosclerosis J A M A *162* 619 1956

4 Adlersberg D & Sobotka H *Pathological manifestations of abnormal cholesterol metabolism In Cholesterol* ed R P Cook p 375 Academic press New York and *London 1958*

5 Ahrens A H Jr Hirsch J Insull W Jr Tsaltas T T Blomstrand R & Peterson M L *Dietary control* of serum lipids in relation to atherosclerosis J A M A *164* 1905 1957

6 Albrink M J & Man E B Serum triglycerides in coronary artery disease Arch int Med *103* 4 1959

7 Albrink M J & Meigs J W Interrelationship between skinfold thickness serum lipids and blood sugar in normal *men* Amer J clin Nutr *15* 255 1964

8 Albrink M J Meigs J W & Granoff M A Weight gain and serum triglycerides in normal men New Engl J Med *266* 484 1962

9 Albrink M J Meigs J W & Man E B Serum lipids hypertension and coronary artery disease Am J Med. *31* 4 1961

10 Anderson J T & Keys A Cholesterol in serum and lipoprotein fractions Clin Chem *2* 145 1956

11 Antonis A & Bersohn I Serum triglyceride levels in South African Europeans and Bantu and in ischaemic heart disease Lancet *I* 998 1960

12 Antonis A Bersohn I Plotkin R Easty D L & Lewis H E The influence of seasonal variation diet and physical activity on serum lipids on young men in Antarctica Amer J clin Nutr *16* 428 1965

13 Aurell E Hjortzberg-Nordlund H & Tibblin G Fetma hos 50-ariga man Somatiska aspekter Lakartidningen *63* 520 1966

14 Berge A & Nicolaysen R Serum cholesterol in middle aged men working in Oslo Scand J Lab clin Invest *15* 284 291 299 1963

15 Blackburn H Brozek J Taylor H L & Keys A Comparison of cardiovascular and related characteristics in habitual smokers and non smokers Ann N Y Acad Sci *90* 277 1960

16 Bøe S Humerfelt S & Wedervang F The blood pressure in a population Acta med scand *157* Suppl 321 1957

17 Bronte Stewart B Cigarette smoking and ischaemic heart disease Brit med J *I* 379 1961

18 Bronte Stewart B Antonis A Eales L & Brock J *Effect of* feeding different fats on serum cholesterol Lancet *I* 521 1956

19 CANTONE A Physical effort and its effect in reducing alimentary hyperlipemia J Sport Med Phys Fit *4* 32 1964

20 CARLSON L A Deponering och mobilisering av fett Sv Läkartidn *57* 2476 1960

21 CARLSON L A Serum lipids in normal men Acta med scand *167* 377 1960

22 CARLSON L A Serum lipids in men with myocardial infarction Acta med scand *167* 399, 1960

23 CARLSON L A Determination of serum triglycerides J atheroscler Res *3* 334 1963

24 CARLSON L A LEVI L & ORO L Plasma lipids and urinary excretion of catecholamines in man during experimentally induced emotional stress and their modification by nicotinic acid J clin Invest *47* 1795, 1968

25 CARLSON L A & MOSSFELDT, F Acute effects of prolonged heavy exercise on the concentration of plasma lipids and lipoproteins in man Acta physiol scand *62* 51 1964

26 COHEN H & GOLDBERG C Effect of physical exercise on alimentary lipemia Brit med J *2* 509 1960

27 CORNFIELD J GORDON T SMITH W W Joint dependence of risk of coronary heart disease on serum cholesterol and systolic blood pressure a discriminant function analysis Fed Proc *21* 58 1962

28 CRAMER K Serum β lipoprotein lipids and protein in normal subjects of different sex and age Acta med scand *171* 413 1962

29 CURRIE A N The cholesterol of blood in malignant disease Brit J exp Path *5* 293 1924

30 DAWBER T R KANNEL W B REVOTSKIE N STOKES J KAGAN A & GORDON T Some factors associated with the development of coronary heart disease Six years follow up experience in the Framingham Study Am J publ Health *49* 1349 1959

31 DOLL R & HILL A B Mortality in relation to smoking Ten years observations of British doctors Brit med J *1* 1460 1964

32 DORN H F Tobacco consumption and mortality from cancer and other diseases Publ Health Rep *75* 581 1959

33 DOYLE J T DAWBER R T KANNEL W B KINCH S H & KAHN H A The relationship of cigarette smoking to coronary heart disease the second report of the combined experience of the Albany, N Y and Framingham Mass studies J A M A *190* 886 1964

34 DOYLE J T HESLIN A S HILLEBOE H E FORMEL P F & KORNS R F A prospective study of degenerative cardiovascular disease in Albany Am J publ Health *47* (part II) 25 1957

35 DOYLE J T HESLIN A S HILLEBOE H E & FORMEL P F Early diagnosis of ischemic heart disease New Engl J Med *261* 1096 1959

36 FELDMAN, E B BENKEL P & NAYAK R V Physiologic factors influencing circulating triglyceride concentration in women J Lab clin Med *62* 437 1963

37 FRIEDMAN M ROSENMAN R H & CARROLL V Changes in the serum cholesterol and blood clotting time in man subjected to cyclic variation of occupational stress Circulation *17* 852, 1958

38 FRISK R WERKO L HOLMGREN A & STRÖM G Stockholm s city health survey 1954 Acta med scand *163* 1 1959

39 FURMAN R H HOWARD R P LAKSHMI K & NORCIA L N The serum lipids and lipoproteins in normal and hyperlipidemic subjects as determined by preparative ultracentrifugation Am J clin Nutr *171* 413 1962

40 GADDUM J H Lognormal distributions Nature *156* 463 1945

41 GOFMAN J W GLAZIER F, TAMPLIN A STRISOWER B & DE LALLA O Lipoproteins coronary heart disease and atherosclerosis Physiol Rev *34*

589 1954
42 GOFMAN J W, LINDGREN F T STRISOWER B DE LALLA O GLAZIER F & TAMPLIN A Cigarette smoking *serum lipoproteins and coronary heart* disease Geriatrics *10* 349 1955
43 GOLDING L A Effects of physical training upon total serum cholesterol level Res Quart amer Ass Health phys Educ *32 499 1961*
44 GRUNDY S M & GRIFFIN A C Effect of periodic mental stress on serum *cholesterol levels Circulation 19* 496 1959
45 GRUNDY S M & GRIFFIN A C Relationship of periodic mental stress to serum lipoprotein and cholesterol levels *J A M A 171* 1794 1959
46 GSELL D & MAYER J Low blood cholesterol associated with high calorie *high saturated fat intakes in a Swiss* Alpine village population Am J clin Nutr *10* 471 1962
47 HAMMOND E C & HORN D The relationship between human smoking *habits and death rates J A M A 166* 1159 1294 1958
48 HANEL H K & DAM H Determination of small amounts of total cholesterol by the Tschugaeff reaction with a note on the determination of lathosterol Acta chem scand *9* 677 1955
49 HAVEL R J & CARLSON L A Serum lipoproteins cholesterol and triglycerides in ischaemic heart disease Metabolism *11 195* 1962
50 HAYES D & NEILL D W Serum cholesterol and triglycerides in ischaemic heart disease Clin Sci 26 185 1964
51 HERNBERG S Serum cholesterol and capacity for physical work *Lancet 2* 441 1964
52 HOLLOSZY J O SKINNER J S TORO G & CURETON T K *Effect* of a six month program of endurance exercise on the serum lipids of middle aged men Am J Cardiol *14* 753 1964
53 JANKELSON O M STEFANIK P A & STARE F D *Serum* lipids and apparent health of Italian American factory workers in a Boston area A four year follow up Am J *clin* Nutr *11* 134 1962
54 JOSEPHSON B & DAHLBERG G Variations *in the cell content and chemical* composition of the human blood due to age sex and season Scand J clin Lab Invest *4* 216 1952
55 KAISER A D & GRAY M S Blood *lipids* in children with fever and rheumatic disease Amer J Dis Child *47* 9 1934
56 KANNEL W B DAWBER T R FRIEDMANN G D GLENNON W E & MCNAMARA P M Risk factors in coronary heart disease An evaluation of several serum lipids as predictors of coronary heart disease The Framingham Study Ann int Med *61* 888 1964
57 KANNEL W B DAWBER T R KAGAN A REVOTSKIE N & STOKES J Factors of risk in the development of coronary heart disease Six year follow up experience The Framingham Study Ann *int* Med *55* 33 1961
58 KARVONEN M J PEKKARINEN M MAETSALA P & RAUTANEN Y Diet and serum cholesterol of lumberjacks Brit J Nutr *15* 157 1961
59 KATZ L N STAMLER J & PICK R Nutrition and atherosclerosis Lea & Febiger Philadelphia 1958
60 KERSHBAUM A BELLET S DICKSTEIN E R & FEINBERG L J Effect *of* cigarette *smoking and nicotine* on serum free fatty acids based on a study in the human subject and the experimental animal Circ Res 9 631 1961
61 KEYS A FIDANZA F & BROZEK J *Cigarette smoking serum-cholesterol* blood pressure and body fatness Observations in Finland Lancet *1* 492 1959
62 KEYS A FIDANZA F SCARDI V BERGAMI G KEYS M H & DiLORENZO F Studies on serum cholesterol and other characteristics of clinically healthy *men in* Naples Arch int Med *93* 328 1954

W H O classification	Number of subjects
322	6
323	1
Diseases of the nervous system	
332	1
345	2
350	1
351	1
352	4
353	3
354	27
355	1
356	1
357	1
362	3
362	3
363	12
364	1
366	5
367	1
368	1
Diseases of the eye	
373	2
375	3
385	6
387	9
388	4
389	11
Diseases of the ear	
390	12
391	23
392	6
395	3
397	3
Rheumatic fever	
400	4
Diseases of the heart and peripheral vessels	
410	3
414	1
421	1
430	1
433	49
434	31
440	41
442	2
446	2
451	1
453	10
456	1
462	10
463	1
467	8
Diseases of the lymphatic system	
468	2
Diseases of the respiratory tract	
470	5
471	2
472	2
474	2
491	3
500	5
502	23
510	1
512	12
513	11
516	3
517	1
525	2
526	5
527	8
Diseases of the digestive system	
536	1
537	1
538	2
540	7
541	21
543	69
544	16
545	3
554	1
562	2
571	6
572	7
573	58
578	2
581	1

W H O classification	Number of subjects
584	33
585	1
586	4
587	1
Diseases of the kidney and urinary tract	
591	1
592	8
600	4
601	1
602	7
603	1
605	5
607	4
609	3
610	32
611	9
Diseases of the genital system including mammary diseases	
614	2
617	13
620	46
621	2
623	2
624	1
625	4
630	51
634	64
635	21
637	33
Puerperal pyelitis	
680	1
Diseases of the skin	
691	2
696	3
701	109
703	2
04	2
05	3
706	61
708	62
710	1
712	1
713	5
714	22
716	11
Diseases of muscles bones and joints	
722	50
723	141
724	2
726	146
730	1
732	2
733	2
734	3
73	71
736	1
737	2
738	29
741	11
42	1
744	21
745	7
748	1
749	6
Malformations	
51	1
753	2
754	3
757	6
758	10
759	1
Symptoms of unspecified cause	
780	20
781	2
82	5
783	2
784	3
785	3
786	3
787	7
788	2
789	65
790	7
791	22
793	46
94	1
95	3

TABLE A 2 Age distribution for men in groups 1–10

Age years	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7		Group 8		Group 9		Group 10	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
15–19	2	0.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.2
20–24	7	0.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	1.6	15	1.2
25–29	17	1.0	0	0.0	0	0.0	2	1.5	0	0.0	0	0.0	0	0.0	3	3.9	10	7.8	33	2.6
30–34	50	2.8	2	1.5	1	2.3	1	0.7	1	4.2	0	0.0	0	0.0	4	5.3	6	4.7	71	5.6
35–39	124	6.9	3	2.2	1	2.3	15	10.9	0	0.0	0	0.0	0	0.0	9	11.8	21	16.3	179	14.1
40–44	311	17.4	27	19.9	5	11.6	38	27.7	0	0.0	4	16.0	0	0.0	19	25.0	32	24.8	289	22.8
45–49	346	19.4	26	19.1	10	23.3	32	23.4	3	12.5	4	16.0	1	100.0	17	22.4	24	18.6	267	21.1
50–54	335	18.7	27	19.9	9	20.9	25	18.2	4	16.7	7	28.0	0	0.0	9	11.8	18	14.0	202	16.0
55–59	294	16.5	20	14.7	8	18.6	15	10.9	11	45.8	4	16.0	0	0.0	10	13.2	9	7.0	126	10.0
60–64	194	10.9	23	16.9	5	11.6	6	4.4	2	8.3	5	20.0	0	0.0	4	5.3	6	4.7	55	4.3
65–69	85	4.8	5	3.7	2	4.7	3	2.2	2	8.3	1	4.0	0	0.0	1	1.3	1	0.8	21	1.7
70–74	20	1.1	3	2.2	2	4.7	0	0.0	1	4.2	0	0.0	0	0.0	0	0.0	0	0.0	5	0.4
75–79	2	0.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	1787		136		43		137		24		25		1		76		129		1266	

N = number of subjects

TABLE A.3 Age distribution for women in groups 1–10

Age years	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 8		Group 9		Group 10	
	N	%	N	%	N	%	N		N	%	N	%	N	%	N	%	N	%
15–19	6	0.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	8	0.8
20–24	20	1.3	0	0.0	2	[illegible]	3	3.1	0	0.0	0	0.0	0	0.0	1	0.3	33	4.1
25–29	27	1.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	6	10.3	3	2.9	37	3.[illegible]
30–34	0	3.4	0	0.0	1	3	0	0.0	0	0.0	1	[illegible]	[illegible]	[illegible]	8	7.[illegible]	32	3.4
35–39	133	8.9	4	4.7	1	3.2	[illegible]	[illegible]	0	0.0	1	4.2	10	17.2	2[illegible]	21.0	112	11.8
40–44	33[illegible]	22.8	13	[illegible]	5	16.1	1[illegible]	19.8	2	10.0	6	2[illegible].0	15	2[illegible].9	2[illegible]	23.8	2[illegible]9	28.3
45–49	302	20.3	11	1[illegible]	8	25.8	27	28.1	0	0.0	4	16.7	[illegible]	8.[illegible]	18	17.1	208	21.9
50–54	2[illegible]8	18.0	18	21.2	6	1[illegible]	18	18.8	2	10.0	3	[illegible]	13	2[illegible].4	17	1[illegible].2	1[illegible]2	16.0
55–59	216	14.[illegible]	2[illegible]	30.6	4	12.[illegible]	11	11.5	0	0.0	4	16.7	4	[illegible]	8	7.[illegible]	[illegible]	7.3
60–64	[illegible]	6.5	7	8.2	3	[illegible]7	8	8.3	1	0.0	3	12.5	1	1.7	3	2.9	18	1.9
65–69	27	1.8	2	2.4	1	3.2	4	4.2	0	0.0	2	8.3	0	0.0	0	0.0	4	0.4
70–74	3	0.2	1	1.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.0
75–79	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	1487		85		31		96		[illegible]		24		58		10[illegible]		949	

N = number of subjects
Group 7 contained no subjects

TABLE A 4 Frequency distribution of concentration of cholesterol for men in the different age classes of group 10

Cholesterol mg/100 ml	Age years																							
	15 19		20—24		25—29		30—34		35—39		40—44		45—49		50—54		55—59		60—64		65—69		70—74	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
000—119	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
120—139	0	0.0	0	0.0	1	3.0	0	0.0	0	0.0	1	0.3	1	0.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
140—159	3	100.0	0	0.0	0	0.0	1	1.4	1	0.6	0	0.0	1	0.4	1	0.5	1	0.8	0	0.0	0	0.0	0	0.0
160—179	0	0.0	3	20.0	0	0.0	2	2.8	4	2.2	3	1.0	2	0.8	6	3.0	1	0.8	1	1.8	0	0.0	0	0.0
180—199	0	0.0	2	13.3	6	18.2	10	14.1	8	4.5	6	2.1	4	1.5	8	4.0	3	2.4	1	1.8	1	4.8	0	0.0
200—219	0	0.0	6	40.0	4	12.1	12	16.9	15	8.4	25	8.7	15	5.6	14	6.9	9	7.1	5	9.1	3	14.3	0	0.0
220—239	0	0.0	0	0.0	7	21.2	11	15.5	31	17.3	30	10.4	29	10.9	25	12.4	8	6.3	5	9.1	2	9.5	1	20.0
240—259	0	0.0	3	20.0	6	18.2	9	12.7	29	16.2	46	15.9	30	11.2	37	18.3	19	15.1	9	16.4	4	19.0	1	20.0
260—279	0	0.0	1	6.7	0	0.0	9	12.7	22	12.3	44	15.2	39	14.6	27	13.4	19	15.1	7	12.7	1	4.8	2	40.0
280—299	0	0.0	0	0.0	2	6.1	5	7.0	26	14.5	53	18.3	40	15.0	28	13.9	21	16.7	5	9.1	4	19.0	1	20.0
300—319	0	0.0	0	0.0	4	12.1	5	7.0	15	8.4	29	10.0	41	15.4	17	8.4	18	14.3	13	23.6	2	9.5	0	0.0
320—339	0	0.0	0	0.0	1	3.0	3	4.2	14	7.8	20	6.9	30	11.2	9	4.5	13	10.3	3	5.4	3	4.3	0	0.0
340—359	0	0.0	0	0.0	0	0.0	2	2.8	3	1.7	12	4.2	14	5.2	8	4.0	4	3.2	2	3.6	0	0.0	0	0.0
360—379	0	0.0	0	0.0	1	3.0	1	1.4	4	2.2	12	4.2	10	3.7	12	5.9	6	4.8	2	3.6	1	4.8	0	0.0
380—399	0	0.0	0	0.0	0	0.0	0	0.0	2	1.1	5	1.7	6	2.2	5	2.5	2	1.6	2	3.6	0	0.0	0	0.0
400—999.9	0	0.0	0	0.0	1	3.0	1	1.4	5	2.8	3	1.0	5	1.9	5	2.5	2	1.6	0	0.0	0	0.0	0	0.0
Total	3		15		33		71		179		289		267		202		126		55		21		5	

N = number of subjects

Table A Frequency distribution of concentration of cholesterol for women in the different age classes of group 10

Cholesterol mg/100 ml	Age years																							
	1 1)		20 24		2 2)		30 34		3 3)		40 44		45 49		0 54		55 59		60 64		65 69		70—74	
	N		N		N		N		N		N		N		N	%	N		N	%	N	%	N	%
00) 11)	0	0 0	2	1	1	2 7	0	0 0	0	0 0	1	0 4	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
120 133	0	0 0	0	0 0	1	7	0	0 0	0	0 0	1	0 4	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
140 1 9	0	0 0	1	2 1	2	5 4	0	0 0	1	0 9	3	1 1	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
160 179	0	0 0	6	12 8	3	8 1	2	6 3	1	0)	4	1 5	1	0 5	2	1 3	1	1	0	0 0	0	0 0	0	0 0
180—191	2	2 0	6	15 4		4	3	9 4	7	6 3	14	2	1	0 5	1	0 7	0	0 0	0	0 0	0	0 0	0	0 0
200 219	1	1	6	15 4		13 5	1	3 1	12	10 7	27	10 0	15	7 2	6	3 9	1	1 5	0	0 0	1	25 0	0	0 0
220 239	3	37 5	6	1 4	6	16 2	4	12	11	9 8	34	12 6	20		11	7 2	5	7 2	0	0 0	0	0 0	0	0 0
240 259	2	2 0	7	17 9	8	21	7	21 9	18	16	39	14 5	31	14 9	16	10 5	7	10 1	1	6	0	0 0	0	0 0
260 274	0	0 0	3	7 7		13			18	16 1	46	17 1	32	15 4	16	10	8	11	1	5 6	0	0 0	0	0 0
280 2))	0	0 0	0	0 0	3	8 1	7	21 9	16	14 3	28	10 4	30	14 4	17	11 2	4	8	3	16 7	1	25 0	0	0 0
300 31)	0	0 0	1	2	0	0 0	1	3 1	18	16 1	32	11	22	10	23	15 1	17	21	2	11	2	50 0	0	0 0
320 339	0	0 0	2	1	0	0 0	1	3 1	3	2 7	3	8 6	3	11 1	2	11	10	14 5	7	38 9	0	0 0	0	0 0
340 3)	0	0 0	0	0 0	0	0 0	1	3 1	3	2 7	4	1	12	5 8	13	8	3	4 3	0	0 0	0	0 0	0	0 0
360 3 9	0	0 0	0	0 0	1	2 7	0	0 0	2	1 8		1 9	8	3 8	8	3		7 2	0	0 0	0	0 0	0	0 0
380—399	0	0 0	0	0 0	0	0 0	0	0 0	1	0 9	3	1 1		2 9	10	6	4	8	1	5	0	0 0	0	0 0
400 9999	0	0 0	0	0 0	0	0 0	0	0 0	1	0)	5	1)	7	3 4	4	2	4	5 8	3	16 7	0	0 0	1	100 0
Total	8		39		37		32		112		269		208		1 2		( )		18		4		1	

N number of subjects

Table A 6 Frequency distribution of concentration of triglycerides for men in the different age classes of group 10

Triglycerides mmol/l	Age years																							
	15 19		20—24		25—29		30—34		35—39		40—44		45—49		50—54		55—59		60—64		65—69		70—74	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0 00 0 19	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
0 20 0 39	0	0 0	1	6 7	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	1	0 5	1	0 8	0	0 0	0	0 0	0	0 0
0 40 0 59	0	0 0	0	0 0	1	3 0	4	5 6	5	2 8	5	1 7	3	1 1	7	3 5	1	0 8	1	1 8	1	4 8	0	0 0
0 60 0 79	1	33 3	4	26 6	1	3 0	3	4 2	15	8 4	17	5 9	12	4 5	8	4 0	6	4 8	1	1 8	2	9 5	2	40 0
0 80 0 99	2	66 7	1	6 7	8	24 2	8	11 3	22	12 3	25	8 7	31	11 6	30	14 9	14	11 1	9	16 4	5	23 8	2	40 0
1 00 1 19	0	0 0	4	26 6	5	15 2	15	21 1	33	18 4	45	15 7	40	15 0	41	20 3	25	19 8	11	20 0	3	14 3	1	20 0
1 20 1 39	0	0 0	1	6 7	7	21 2	14	19 7	28	15 6	39	13 5	36	13 5	28	13 9	17	13 5	11	20 0	3	14 3	0	0 0
1 40—1 59	0	0 0	2	13 3	1	3 0	10	14 1	23	12 8	51	17 6	44	16 5	21	10 4	21	16 7	8	14 5	2	9 5	0	0 0
1 60 1 79	0	0 0	0	0 0	3	10 0	6	8 5	12	6 7	33	11 4	26	9 7	15	7 4	20	15 9	9	16 4	2	9 5	0	0 0
1 80—1 99	0	0 0	1	6 7	3	10 0	4	5 6	16	8 9	17	5 9	10	3 7	11	5 4	9	7 1	3	5 5	1	4 8	0	0 0
2 00—2 19	0	0 0	0	0 0	1	3 0	2	2 8	8	4 5	16	5 5	23	8 6	8	4 0	3	2 4	1	1 8	0	0 0	0	0 0
2 20—2 39	0	0 0	0	0 0	1	3 0	0	0 0	3	1 7	12	4 2	14	5 2	11	5 4	3	2 4	1	1 8	1	4 8	0	0 0
2 40—2 59	0	0 0	0	0 0	0	0 0	1	1 4	6	3 4	6	2 1	8	3 0	6	3 0	2	1 6	0	0 0	1	4 8	0	0 0
2 60—2 79	0	0 0	0	0 0	0	0 0	2	2 8	1	0 6	2	0 7	9	3 4	7	3 5	3	2 4	0	0 0	0	0 0	0	0 0
2 80—2 99	0	0 0	1	6 7	1	3 0	0	0 0	0	0 0	5	1 7	3	1 1	4	2 0	0	0 0	0	0 0	0	0 0	0	0 0
3 00—9 999	0	0 0	0	0 0	1	3 0	2	2 8	7	3 9	16	5 5	8	3 7	4	2 0	1	0 8	0	0 0	0	0 0	0	0 0
Total	3		15		33		71		179		289		267		202		126		55		21		5	

N — number of subjects

TABLE A 7 Frequency d str bution of concer tra on of tr glycer les for omen in the d fferent age classes of group 10

Triglycerides mmol/l	Age years 1 19		20 24		25 29		30 34		35 39		40 44		4 49		0— 4		55—59		60—64		65—69		70—74	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0 00 0 19	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	1	0 4	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
0 20 0 39	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	1	0 4	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
0 40 0 59	0	0 0	6	[illegible] 4	4	10 8	[illegible]	1	3	2 7	18	[illegible] 7	8	3 8	2	1 3	0	0 0	0	0 0	0	0 0	0	0 0
0 60—0 79	1	12	[illegible]	[illegible]		13	[illegible]	[illegible]	1	13 4	44	[illegible]	31	[illegible]	13	8 [illegible]	5	1 3	2	11 1	1	2 0	0	0 0
0 80 0 99	[illegible]	0 0	8	20	13	3 [illegible]	11	34 4	3	31 3	60	2 3	37	17 8	31	20 4	1	21 7	3	16 7	0	0 0	0	0 0
1 00 1 19	3	37 5	11	28 2	3	8 1	1	3 1	26	3 2	71	28 3	[illegible]	20 2	29	[illegible]	22	31 [illegible]	[illegible]	33 3	1	2 0	0	0 0
1 20 1 39	0	0 0	6	15 4	4	10 8	4	12	13	[illegible]	26	9 7	23	13 9	47	17 8	16	23 2	1	[illegible]	2	0 0	1	100 0
1 40 1 59	0	0 0	2	5 1	8	21 [illegible]	2	[illegible] 3	8	7 1	13	7 1	17	8 2	17	11 2		7 2	3	16 7	0	0 0	0	0 0
1 60 1 79	0	0 0	0	0 0	0	0 0	1	3 1	1	3 [illegible]	10	3 7	13	6 3	[illegible]	[illegible] 3	2	2 [illegible]	2	11 1	0	0 0	0	0 0
1 80 1 99	0	0 0	0	0 0	0	0 0	1	3 1	[illegible]	1 8	[illegible]	1 9	13	[illegible] 3	11	7 [illegible]	3	[illegible] 3	0	0 0	0	0 0	0	0 0
2 00 2 19	0	0 0	0	0 0	0	0 0	0	0 0	2	1 8		[illegible]	8	3 8	2	1 3	0	0 0	1	5 [illegible]	0	0 0	0	0 0
2 20 2 39	0	0 0	0	0 0	0	0 0	2	[illegible] 3	1	0 [illegible]	1	0 4	8	3 9	2	1 3	0	0 0	0	0 0	0	0 0	0	0 0
2 40—2 59	0	0 0	0	0 0	0	0 0	1	3 1	1	0 [illegible]	2	0 7	1	0 [illegible]	1	0 7	1	1 4	0	0 0	0	0 0	0	0 0
2 60 2 79	0	0 0	0	0 0	0	0 0	0	0 0	1	0 9	0	0 0	0	0 0	1	0 7	0	0 0	0	0 0	0	0 0	0	0 0
2 80—2 99	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
3 00 9 99	0	0 0	0	0 0	0	0 0	0	0 0	1	0 [illegible]	1	0 4	1	0 [illegible]	1	0 7	[illegible]	2 9	0	0 0	0	0 0	0	0 0
Total	8		3[illegible]		37		32		112		2[illegible]		208		1 2		[illegible]		18		4		1	

N → number f subje ts

Table A 8 Frequency distribution of log concentration of triglycerides for men in the different age classes of group 10

Triglycerides (g/litre)	Age (years) 15—19		20—24		25—29		30—34		35—39		40—44		45—49		50—54		55—59		60—64		65—69		70—74	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
000 0 (01	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
0 (00 0 ,1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
0 0 0 01	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
0 00 04 1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0
0 4 0 0 401	0	0.0	1	6.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8	0	0.0	0	0.0	0	0.0
0 400 0 351	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.3	0	0.0	1	0.5	1	0.8	0	0.0	0	0.0	0	0.0
0 350 0 301	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	1	0.4	2	1.0	0	0.0	0	0.0	1	4.8	0	0.0
0 300 0 251	0	0.0	0	0.0	1	3.0	3	4.2	3	1.7	3	1.0	1	0.4	3	1.5	0	0.0	1	1.8	0	0.0	0	0.0
-0 2 0 0 201	0	0.0	1	6.7	0	0.0	0	0.0	4	2.2	3	1.0	2	0.7	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0
0 200 0 151	0	0.0	1	6.7	0	0.0	1	1.4	6	3.4	4	1.4	4	1.5	4	2.0	2	1.6	0	0.0	2	9.5	1	20.0
0 150 0 101	1	33.3	2	13.3	1	3.0	2	2.8	7	3.9	11	3.8	7	2.6	4	2.0	4	3.2	1	1.8	0	0.0	1	20.0
0 100 0 051	2	66.7	1	6.7	3	9.1	7	9.9	13	7.3	6	2.1	11	4.1	16	7.9	2	1.6	6	10.9	1	4.8	2	40.0
0 0 0 0 001	0	0.0	0	0.0	5	15.2	1	1.4	9	5.0	19	6.6	20	7.5	14	6.9	12	9.5	3	5.5	4	19.0	0	0.0
0 000 0 049	0	0.0	3	20.0	4	12.1	11	15.5	22	12.2	33	11.4	27	10.1	24	11.9	18	14.3	6	10.9	3	14.3	0	0.0
0 050— 0 099	0	0.0	1	6.7	4	12.1	8	11.3	20	11.2	24	8.3	25	9.4	26	12.9	11	8.7	8	14.5	2	9.5	1	20.0
0 100— 0 149	0	0.0	3	20.0	4	12.1	12	16.9	19	10.6	35	12.1	29	10.9	22	10.9	18	14.3	8	14.5	2	9.5	0	0.0
0 150— 0 199	0	0.0	0	0.0	1	3.0	8	11.3	22	12.2	40	13.8	38	14.2	18	8.9	16	12.7	8	14.5	1	4.8	0	0.0
0 200— 0 249	0	0.0	0	0.0	3	9.1	5	7.0	12	6.7	34	11.8	25	9.4	14	6.9	16	12.7	6	10.9	2	9.5	0	0.0
0 250— 0 299	0	0.0	1	6.7	3	9.1	5	7.0	17	9.5	19	6.6	12	4.5	12	5.9	13	10.3	6	10.9	1	4.8	0	0.0
0 300— 0 349	0	0.0	0	0.0	2	6.1	2	2.8	9	5.0	22	7.6	29	10.9	13	6.4	4	3.2	2	3.6	0	0.0	0	0.0
0 350— 0 399	0	0.0	0	0.0	0	0.0	1	1.4	8	4.5	11	3.8	16	6.0	10	5.0	3	2.4	0	0.0	1	4.8	0	0.0
0 400— 0 449	0	0.0	0	0.0	0	0.0	2	2.8	1	0.6	4	1.4	10	3.7	9	4.5	4	3.2	0	0.0	1	4.8	0	0.0
0 450— 0 499	0	0.0	1	6.7	1	3.0	2	2.8	0	0.0	6	2.1	3	1.1	4	2.0	0	0.0	0	0.0	0	0.0	0	0.0
0 500— 7 000	0	0.0	0	0.0	1	3.0	0	0.0	7	3.9	14	4.8	7	2.6	4	2.0	1	0.8	0	0.0	0	0.0	0	0.0
Total	3		15		33		71		179		289		267		202		126		55		21		5	

N = number of subjects

T sn \ 9 Ireque cy d s r l n of log conce trat a C tr glycer des f r wo en n tl e d ffere t age cl sses of group 10

I gl c r l s		Ag yer s																							
I g l l		1 19		20 21		2 23		30 34		3 41		40—44		4 49		0—54		5 59		60—64		(—69		70—74	
		N		N		N		N		N		N		N		N		N		N		N		N	
2 001	0 101	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	1	0 4	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
0 00	0 5 1	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
0 0	0 01	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	1	0 4	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
0 00	0 4 1	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
0 4 0	1 301	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
0 400	0 3 1	0	0 0	0	0 0	0	0 0	1	3 1	0	0 0	4	1	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
0 3 0	0 301	0	0 0	1	(	4	10 8	1	3 1	1	0 9	1	1	3	1 4	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
0 300	0 2 1	0	0 0	3	7 7	0	0 0	2	6 3	1	0 9	(	2 2	0	0 0	1	0 7	(	0 0	0	0 0	0	0 0	0	0 0
0 250	0 01	1	12	4	10 3	1	2 7	1	3 1	4	3 1	11	1 1	12	8	1	0 7	0	0 0	1	(	0	0 0	0	0 0
0 200	0 1 1	0	0 0	1	1 (	2	4	3	1 4	7	6 3	11	2	10	1 0	5	3 3	0	0 0	0	0 0	0	0 0	0	0 0
(1 0	0 101	0	0 0	3	7 7	2	1	1	3 1	5	4 5	23	8 (	14	(7	8	5 3	3	4 3	1	1	1 ?	0	0	0 0
0 100	0 0 1	3	37		12 8	8	21 (	8	25 0	1	13 4	37	11 9	1	11	11	7 2	(	8 7	0	0 0	0	0 0	0	0 0
0 0 0	0 101	1	12	3	7 7		13	3	1 4	20	17 1	8	10 4	1	(3	20	13 2	9	13 0	3	11 7	0	0 0	0	0 0
0 000	0 019	3	37	(	1 4	1	2 7	1	3 1	3	20	0	18 (	20	13	18	11 8	11	20 3	5	27 8	1	2 0	0	0 0
0 0 0	0 019	0	0 0		1 1	4	10 8	1	3 1	4	3 (	3	13 0	21	10 1	21	13 8	14	0 1	1	1	1	25 0	0	0 0
0 100	0 14	0	0 0		12 8	4	10 8	3	9 4	12	10 7	18	(7	22	10 (	1	1	11	15 9	1	(	1	2 0	1	100 0
0 1 0	0 1 9	0	(0	2	5 1		11 2	2	(3	7	(5	18	(7	1	7 2	1	19	4	8	3	1(7	0	0 0	0	0 0
0 00	0 24	0	0 0	0	(0	0	0 0	0	0 0	5	1	9	3 3	12	8	13	8 (	2	9	1	1	0	0 0	0	0 0
0 2 0	(29	0	0 0	0	0 0	0	0 0	2	(3	2	1 8	(	2 2	1(	7 7	13	8 (	3	1 1	1	1	0	0 0	0	0 0
0 300	0 31	0	0 0	0	0 0	0	0 0	0	0 0	2	1 8		1	9	(3	3	2 0	0	0 0	1	(	0	0 0	0	0 0
0 3 0	0 3 1	0	0 0	0	0 0	0	0 0	2	(3	2	1 8	2	0 7	8	3 8	1	0 7	0	0 0	0	0 0	0	0 0	0	0 0
0 400	0 41	0	0 0	0	0 0	0	0 0	1	3 1	1	1 1	1	0 1	0	0 0	2	1 3	1	1 4	0	0 0	0	0 0	0	0 0
0 1 0	0 41	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0	0	0 0
0 00—	7 010	0	0 0	0	0 0	0	0 0	0	0 0	1	0 9	1	0 4	1	0	1	0 7	2	2 9	0	0 0	0	0 0	0	0 0
Total		8		3		37		32		112		2(		208		1 2		(		18		4		1	

N ber f sul je ts

TABLE A 10 Concentration of cholesterol and index of skewness of distribution of values within each age class of group 10

Age years	Men						Women					
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	$s_{\bar{x}}$ mg/100 ml	g	s_g	N	$\bar{x}$ mg/100 ml	s mg/100 ml	$s_{\bar{x}}$ mg/100 ml	g	s_g
15–19	3	152.7	5.8	3.3	−1.73	1.22	8	223.5	25.1	8.9	−0.40	0.75
20–24	15	213.3	31.9	8.2	0.26	0.58	39	218.4	48.5	7.8	0.17	0.38
25–29	33	215.8	59.4	10.3	1.24**	0.41	37	238.7	53.0	8.7	0.04	0.39
30–34	71	247.5	52.2	6.2	0.68*	0.28	32	253.8	43.7	7.7	0.03	0.41
35–39	169	269.3	55.4	4.1	1.00***	0.18	112	266.6	51.9	4.9	1.19***	0.23
40–44	209	266.6	49.8	2.9	0.39**	0.17	269	266.2	53.7	3.3	0.40**	0.15
45–49	267	285.7	51.5	3.2	0.12	0.15	208	288.6	54.4	3.8	0.83***	0.17
50–54	202	274.4	56.7	4.0	0.47	0.17	152	303.0	55.4	4.5	0.20	0.20
55–59	126	282.8	49.9	4.4	0.12	0.22	69	309.5	59.7	7.2	0.59	0.29
60–64	55	278.2	50.3	6.8	0.27	0.32	18	330.8	52.5	12.4	0.60	0.54
65–69	21	273.8	48.0	10.5	0.21	0.50	4	278.5	52.7	26.3	−1.79	1.01
70–74	5	259.6	23.3	10.4	−0.73	0.91	1	492.0	—	—	—	—

N = number of subjects, $\bar{x}$ = mean value, s = standard deviation, $s_{\bar{x}}$ = standard error of the mean, g = index of skewness, s_g = standard error of g

TABLE A 11 Concentration of triglycerides and index of skewness of distribution of values within each age class of group 10

Age years	Men						Women					
	N	$\bar{x}$ mmole/l	s mmole/l	s mmole/l	g	s_g	N	$\bar{x}$ mmole/l	s mmole/l	s mmole/l	g	s_g
15–19	3	0.80	0.06	0.03	1.67	1.22	8	0.91	0.17	0.06	0.17	0.75
20–24	15	1.15	0.60	0.16	1.63**	0.58	33	0.96	0.29	0.05	0.04	0.38
25–29	33	1.39	0.62	0.11	1.70***	0.41	37	1.00	0.34	0.06	0.19	0.39
30–34	71	1.36	0.54	0.06	1.18***	0.28	35	1.09	0.55	0.10	1.27**	0.11
35–39	175	1.44	0.67	0.05	1.80***	0.18	112	1.11	0.60	0.06	5.02***	0.23
40–44	289	1.58	0.73	0.04	1.92***	0.14	269	1.07	0.41	0.03	1.75***	0.15
45–49	267	1.58	0.75	0.05	2.16***	0.15	208	1.23	0.49	0.03	1.05***	0.17
50–54	202	1.48	0.69	0.05	1.57***	0.17	152	1.28	0.14	0.04	1.31***	0.20
55–59	126	1.42	0.49	0.04	0.99***	0.22	69	1.27	0.57	0.07	3.73***	0.29
60–64	55	1.35	0.36	0.05	0.18	0.32	18	1.22	0.38	0.09	0.72	0.51
65–69	21	1.26	0.34	0.12	0.95	0.50	4	1.06	0.27	0.14	0.66	1.01
70–74	5	0.86	0.20	0.09	1.24	0.91	1	1.38			—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation s = standard error of the mean g = index of skewness s_g = standard error of g

TABLE A 12 Log concentration of triglycerides and index of skewness of distribution of values within each age class of group 10

Age years	Men						Women					
	N	$\bar{x}$ log mmole/l	s log mmole/l	$s_{\bar{x}}$ log mmole/l	g	s_g	N	$\bar{x}$ log mmole/l	s log mmole/l	$s_{\bar{x}}$ log mmole/l	g	s_g
15–19	3	0 096	0 030	0 018	−1 68	1 22	8	−0 051	0 087	0 031	−0 92	0 75
20–24	15	0 011	0 214	0 056	−0 01	0 58	39	−0 040	0 138	0 022	−0 42	0 38
25–29	33	0 108	0 169	0 030	0 55	0 41	37	−0 025	0 157	0 026	−0 42	0 39
30–34	71	0 101	0 168	0 020	−0 10	0 28	32	0 010	0 201	0 036	0 38	0 41
35–39	179	0 118	0 181	0 014	0 29	0 18	112	0 025	0 155	0 015	1 23***	0 23
40–44	289	0 159	0 178	0 011	0 25	0 14	269	−0 003	0 169	0 010	−0 94***	0 15
45–49	267	0 162	0 175	0 011	0 31*	0 15	208	0 057	0 168	0 012	0 10	0 17
50–54	202	0 127	0 190	0 013	0 01	0 17	152	0 082	0 141	0 011	0 18	0 20
55–59	126	0 126	0 152	0 014	−0 47*	0 22	69	0 077	0 136	0 016	1 86***	0 29
60–64	55	0 103	0 124	0 017	−0 48	0 32	18	0 066	0 134	0 032	0 09	0 54
65–69	21	0 065	0 181	0 040	0 02	0 50	4	0 014	0 120	0 060	−1 00	1 01
70–74	5	−0 074	0 096	0 043	0 81	0 91	1	0 139	—	—	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean g = index of skewness s_g = standard error of g

TABLE A 13 The regress ons of concentrat on of choles erol (n g per 100 ml) and tr glycer des (n mole per l) and of log concentrat on of tr glycer des (log mmole per l) on age (years) for men n group 10

Age years	Cholesterol Age					Tr glycer des Age				log Tr glycer des Age			
	N	b	s	s_b	t	b	s	s_b	t	b	s	s_b	t
15 19	3	10 00				0 010	0 071	0 081	0 461	0 022	0 038	0 0177	0 468
15 24	18	8 02*	32 77	3 31	2 420	0 0.7	0 564	0 057	1 000	0 014	0 202	0 0704	0 709
1.—29	51	7 80**	1 57	2 44	3 198	0 06 *	0 84	0 028	2 383	0 021*	0 178	0 0084	2 496
15—34	122	3 40**	521	1 15	2 9 0	0 019	0 567	0 01?	1 561	0 007	0 174	0 0038	1 900
15 39	301	3 32***	.4 27	0 (4	5 19	0 016*	0 (30	0 007	2 (33	0 004*	0 178	0 0021	2 323
1. 44	.90	2 71***	52 27	0 40	6 7 8	0 01]***	0 181	0 005	3 721	0 005***	0 178	0 0013	1 321
1 49	8 7	2 33***	52 14	0 29	8 109	0 014***	0 703	0 004	3 00	0 001***	0 178	0 0009	4 4 7
15 4	10.9	1 48***	3 .(	0 23	6 386	0 007*	0 02	0 003	2 38	0 003**	0 181	0 0007	2 7.9
1 — 9	1185	1 25***	3 24	0 19	(447	0 003	0 (84	0 002	1 3 0	0 001*	0 178	0 0006	1 99
15 64	1240	1 08***	53 19	0 18	(11(	0(1	0 174	0 002	0 4(3	0 0)0	0 177	0 000	1 2 7
1. 69	12(1	0 98***	.3 1	0 17	5 818	0 000	0 173	0 (02	0 029	0 000	0 177	0 000.	0 (3(
15—74	12((	0 93***	53 14	0 17	619	0 001	0 173	0 00	0 40	0 000	0 177	0 000	0 147
15—79	1266	0 93***	53 14	0 17	(19	0 001	0 73	0 002	0 40	0 000	0 177	0 000	0 147
20—79	1263	0 8 ***	52 39	0 17	0 0	0 001	0 673	0 00	0 680	J 000	0 177	0 000.	0 19(
25 79	1248	0 69***	2 98	0 17	4 000	0 003	0 (72	0 0 2	1 73	0 000	0 17(	0 0005	0 990
30—79	1215	0 56**	?2 7.	0 19	3 031	0 004	0 673	0 002	(74(	0 000	0 171	0 0006	1 1 4
35 79	1144	0 23	2 48	0 21	1 109	0 007**	0 (79	0 003	2 723	0 001*	0 17(	0 0006	2 438
40 7.	965	0 12	51 79	0 25	0 488	0 01]***	0 17(	0 003	4 3	0 003***	0 171	0 0003	1 270
45 79	67(	0 (0	.2 0	0 3(	1 (81	0 020***	0 (0	0 004	4 1 0	0 00 ***	0 172	0 0011	1 313
50 79	409	0 00	.3 13	0	0 (01	0 01]**	0 92	0 00	2 0 2	0 001*	0 1 0	0 0017	2 11
. —79	07	1 07	49 2	0 8	1 1.	0 0 0*	0 1(2	0 008	0	0 007**	0 148	0 (02	2 7 2
60—7)	81	1 03	48 48	1 (8	0 (09	0 0 7	0 10.	0 014	1 .17	0 01 ?*	0 140	0 0018	2 18
(5—79	(	—1 7(	15 01	3 ?	0 477	0 0	0 195	0 041	1 70.	0 02	0 1(8	0 0137	1 82.
70—79	.	20	. 32	8 3.	0 (2	0 09(	0 1(3	0 0 4	1 89	0 017	0 07.	0 0?47	1 .08

N = nun ber of subjects b = reg ess on coeff er t s s a dar l de at on fr r gress s_b stand rd de at n f he regress o coeff c e t
t = b s_b

TABLE A 14 The regressions of concentration of cholesterol (mg per 100 ml) and triglycerides (mmole per l) and of log concentration of triglycerides (log mmole per l) on age (years) for women in group 10

Age years	Cholesterol Age					Triglycerides Age				log Triglycerides Age			
	N	b	s	s_b	t	b	s	s_b	t	b	s	s_b	t
15–19	8	4.42	26.34	7.60	0.580	−0.026	0.181	0.052	−0.495	−0.015	0.091	0.0265	−0.587
15–24	47	3.29	45.17	3.18	1.033	−0.002	0.271	0.019	−0.111	−0.002	0.131	0.0092	−0.232
15–29	84	1.94	18.53	1.55	1.254	0.005	0.303	0.010	0.538	0.001	0.142	0.0045	0.285
15–34	116	2.98**	47.22	0.93	3.216	0.013	0.384	0.008	1.765	0.003	0.150	0.0031	0.994
15–39	228	3.09***	49.41	0.50	6.187	0.012*	0.503	0.005	2.387	0.004**	0.157	0.0015	2.722
15–44	497	2.35***	51.77	0.34	6.900	0.005	0.458	0.003	1.621	0.001	0.164	0.0010	1.654
15–49	705	2.61***	52.61	0.27	9.626	0.010***	0.470	0.002	3.970	0.003***	0.166	0.0008	4.094
15–54	857	2.73***	53.16	0.23	12.038	0.011***	0.465	0.002	5.593	0.004***	0.162	0.0006	6.167
15–59	926	2.68***	53.62	0.20	13.101	0.011***	0.473	0.002	5.854	0.004***	0.160	0.0006	6.701
15–64	914	2.69***	53.61	0.20	13.735	0.010***	0.472	0.002	5.824	0.003***	0.159	0.0005	6.736
15–69	918	2.63***	53.71	0.19	13.541	0.010***	0.471	0.002	5.693	0.003***	0.159	0.0005	6.621
15–74	919	2.67***	53.89	0.19	13.811	0.010***	0.471	0.002	5.715	0.003***	0.159	0.0005	6.659
15–79	919	2.67***	53.89	0.19	13.811	0.010***	0.471	0.002	5.715	0.003***	0.159	0.0005	6.659
20–79	911	2.72***	54.06	0.20	13.495	0.010***	0.473	0.002	5.194	0.003***	0.160	0.0005	6.487
25–79	902	2.70***	54.35	0.24	11.107	0.010***	0.479	0.002	4.741	0.004***	0.161	0.0007	5.812
30–79	865	2.63***	54.43	0.27	9.750	0.010***	0.485	0.002	4.229	0.004***	0.161	0.0007	5.405
35–79	833	2.72***	54.80	0.29	9.219	0.011***	0.482	0.003	4.112	0.004***	0.159	0.0008	5.122
40–79	721	3.03***	55.19	0.35	8.561	0.014***	0.460	0.003	4.687	0.005***	0.159	0.0010	5.389
45–79	452	2.19***	56.08	0.56	3.909	0.005	0.483	0.005	1.062	0.002	0.153	0.0015	1.546
50–79	244	1.74	57.49	0.94	1.856	0.001	0.474	0.008	0.110	0.000	0.138	0.0022	0.070
55–79	92	2.77	60.84	1.95	1.418	−0.001	0.530	0.017	−0.083	0.000	0.134	0.0043	0.071
60–79	23	−0.14	66.18	5.03	0.027	0.022	0.363	0.028	0.806	0.008	0.129	0.0098	0.879
65–79	5	40.95	66.50	15.34	2.669	0.110	0.159	0.037	3.006	0.015	0.076	0.0176	2.564

N = number of subjects b = regression coefficient s = standard deviation from regression s_b = standard deviation of the regression coefficient
t = b/s_b

TABLE A 15 Concentration of cholesterol and triglycerides and log concentration of triglycerides for men in group I

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	$s_{\bar{x}}$ mg/100 ml	$\bar{x}$ mmol/l	s mmol/l	$s_{\bar{x}}$ mmol/l	$\bar{x}$ log mmol/l	s log mmol/l	$s_{\bar{x}}$ log mmol/l
15—19	2	187.0	17.7	9.0	0.94	0.04	0.03	—0.030	0.016	0.01[illegible]
20—24	7	203.6	51.5	19.5	1.22	0.23	0.09	0.0[illegible]9	0.0[illegible]8	0.030
25—29	17	236.5	39.[illegible]	9.5	1.15	0.35	0.08	0.04	0.115	0.078
30—34	50	26[illegible].7	60.9	8.6	1.44	0.81	0.12	0.110	0.194	0.0[illegible]
35—39	124	273.3	55.3	5.0	1.48	0.73	0.07	0.130	0.185	0.01
40—44	311	27[illegible].3	61.8	3.5	1.66	1.07	0.06	0.16[illegible]	0.21[illegible]	0.01[illegible]
45—49	346	284.6	67.4	3.4	1.71	0.93	0.05	0.183	0.201	0.011
50—54	335	288.9	67.6	3.4	1.65	0.84	0.05	0.1[illegible]	0.19[illegible]	0.011
55—59	294	284.0	63.1	3.7	1.[illegible]3	0.96	0.0[illegible]	0.191	0.195	0.011
60—64	194	[illegible]81.9	61.6	4.4	1.57	0.8	0.06	0.148	0.198	0.014
65—69	85	279.7	47.5	5.1	1.47	0.58	0.06	0.136	0.161	0.018
70—74	20	240.1	50.4	11.3	1.55	0.60	0.13	0.15	0.19	0.044
75—79	2	264.0	56.6	40.0	1.54	1.[illegible]4	0.88	0.101	0.399	0.28

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

TABLE A 16 Concentration of cholesterol and triglycerides and log concentration of triglycerides for women in group I

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	$s_{\bar{x}}$ mg/100 ml	$\bar{x}$ mmol/l	s mmol/l	$s_{\bar{x}}$ mmol/l	$\bar{x}$ log mmol/l	s log mmol/l	$s_{\bar{x}}$ log mmol/l
15—19	6	238.5	50.4	20.6	0.83	0.26	0.10	-0.101	0.13[illegible]	0.056
20—24	20	217.2	4[illegible]	10.7	1.01	0.37	0.08	—0.0[illegible]3	0.158	0.035
25—29	[illegible]7	[illegible]27.3	45.6	8.8	0.93	0.39	0.08	—0.065	0.1[illegible]2	0.033
30—34	50	243.4	[illegible]9.4	7.0	1.0	0.30	0.04	0.013	0.121	0.01
35—39	133	266.6	5[illegible].1	4.5	1.16	0.50	0.04	0.0[illegible]6	0.1[illegible]9	0.016
40—44	339	274.6	68.4	3.7	1.21	0.59	0.03	0.046	0.175	0.010
45—49	30	286.2	54.1	3.1	1.35	0.[illegible]7	0.04	0.089	0.18[illegible]	0.010
50—54	268	298.7	58.6	3.6	1.34	0.54	0.03	0.095	0.1[illegible]2	0.011
55—59	216	313.7	65.1	4.4	1.4[illegible]	0.53	0.04	0.1[illegible]3	0.154	0.011
60—64	96	309.8	55.0	[illegible].6	1.43	0.56	0.06	0.119	0.180	0.018
65—69	27	[illegible]94.8	61	11.8	1.[illegible]8	0.87	0.17	0.199	0.2[illegible]1	0.043
[illegible]0—[illegible]4	3	29[illegible].0	[illegible]0.4	11.8	1.50	0.8[illegible]	0.47	0.134	0.2[illegible]	0.131

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

TABLE A 17 Concentration of cholesterol and triglycerides and log concentration of triglycerides for men in group 2

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	$s_{\bar{x}}$ mg/100 ml	$\bar{x}$ mmole/l	s mmole/l	$s_{\bar{x}}$ mmole/l	$\bar{x}$ log mmole/l	s log mmole/l	$s_{\bar{x}}$ log mmole/l
15—19	0	—	—	—	—	—	—	—	—	—
20—24	0	—	—	—	—	—	—	—	—	—
25—29	0	—	—	—	—	—	—	—	—	—
30—34	2	266.0	8.5	6.0	1.24	0.41	0.29	0.081	0.146	0.103
35—39	3	280.3	66.4	38.4	2.39	1.65	0.95	0.294	0.348	0.201
40—44	27	309.3	126.7	24.4	2.07	1.22	0.23	0.241	0.237	0.046
45—49	2	297.2	63.8	12.5	1.60	0.82	0.16	0.160	0.198	0.039
50—54	27	279.4	53.9	10.4	1.47	0.48	0.09	0.145	0.139	0.027
55—59	20	283.8	6[illegible].8	14.7	1.58	1.02	0.23	0.138	0.217	0.049
60—64	23	28[illegible].2	68.8	14.3	1.43	0.54	0.11	0.129	0.157	0.033
65—69	5	284.6	44.0	19.7	1.39	0.51	0.23	0.119	0.161	0.072
70—74	3	257.3	7.6	4.4	1.18	0.24	0.14	0.067	0.088	0.051

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

TABLE A 18 Concentration of cholesterol and triglycerides and log concentration of triglycerides for women in group 2

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	$s_{\bar{x}}$ mg/100 ml	$\bar{x}$ mmole/l	s mmole/l	$s_{\bar{x}}$ mmole/l	$\bar{x}$ log mmole/l	s log mmole/l	$s_{\bar{x}}$ log mmole/l
15—19	0		—	—	—	—	—	—	—	—
20—24	0		—	—	—	—	—	—	—	—
25—29	0	—	—	—	—	—	—	—	—	—
30—34	0			—	—	—	—	—	—	—
35—39	4	264.0	32.3	16.1	1.52	0.46	0.23	0.165	0.133	0.067
40—44	13	2[illegible]8.8	32.2	8.9	1.23	0.37	0.10	0.072	0.134	0.037
45—49	14	28[illegible].[illegible]	[illegible]0.7	13.5	1.39	0.39	0.10	0.127	0.113	0.030
50—54	18	30[illegible].6	67.5	15.9	1.22	0.47	0.11	0.0[illegible]9	0.158	0.037
55—59		3[illegible]0.3	68.8	13.5	1.[illegible]0	0.[illegible]1	0.10	0.147	0.167	0.033
60—64		313.1	44.8	16.9	1.05	0.32	0.12	0.003	0.140	0.0[illegible]3
65—69		339.0	123.0	87.0	1.94	1.47	1.04	0.214	0.307	0.260
70—74	1	340.0			1.14	—	—	0.0[illegible]	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

Table A 19 Concentration of cholesterol and triglycerides and log concentration of triglycerides for men in group 3

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$
15—19	0	—	—	—	—		—	—	—	—
20—24	0	—	—	—	—		—		—	—
25—29	0	—	—	—		—	—	—	—	—
30—34	1	213 0	—	—	0 86	—	—	0 066	—	—
35—39	1	280 0	—	—	3 00		—	0 4	—	—
40—44	5	209 2	65.1	29 6	1 4	0.5	0.23	0 14	0 141	0 063
45—49	10	2 58	43 1	13 6	1 0	0 95	0 30	0 180	0 17	0 06
50—54	9	280 1	20 6	6 9	1 5	0 60	0 20	0 166	0 153	0 053
55—59	8	263 6	43 2	15 3	1 39	0 4	0 16	0 114	0 1 9	0 063
60—64	5	230.2	64 5	28 8	1 51	0 64	0.29	0 152	0 164	0 0 4
65—69	2	29 0	12 7	9 0	1 51	0 69	0 49	0 154	0 06	0 146
70—74	2	319 5	105 4	4 5	1 34	0 58	0 41	0 105	0 194	0 13

N = number of subjects x mean value s = standard deviation $s_{\bar{x}}$ standard error of the mean

Table A 20 Concentration of cholesterol and triglycerides and log concentration of triglycerides for women in group 3

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$
15—19	0	—	—	—	—	—	—	—	—	—
20—24	2	210 0	8 5	6 0	0.81	0 14	0 10	—0 095	0 0 6	0 054
25—29	0	—		—			—	—	—	—
30—34	1	203 0	—	—	1 02			0 008	—	—
35—39	1	241 0		—	0 80	—		—0 09	—	—
40—44	5	319 4	98	44 1	1 15	0 9	0 13	0 051	0 102	0 046
45—49	8	313 4	108 9	38 5	1 0	0 11	0 04	0 00	0 05	0 019
50—54	6	33 5	40 3	16 5	1 4	0.55	0.22	0 130	0 16	0 083
55—59	4	345 0	105 5	5 8	1 4	0 40	0 20	0 230	0 103	0 054
60—64	3	69 0	48 1	2 8	1 1	0 15	0 08	0 066	0 050	0 079
65—69	1	45 0	00 0	00 0	1 55	—	—	0 130	—	—
70—74	0	—		—	—	—	—	—	—	—

N = number of subjects x = mean value s standard deviation, $s_{\bar{x}}$ = standard error of the mean

TABLE N 21 Concentration of cholesterol and triglycerides and log concentration of triglycerides for men in group 4

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	$s_{\bar{x}}$ mg/100 ml	$\bar{x}$ mmole/l	s mmole/l	$s_{\bar{x}}$ mmole/l	$\bar{x}$ log mmole/l	s log mmole/l	$s_{\bar{x}}$ log mmole/l
15—19	0	—	—	—	—	—	—	—	—	—
20—24	0	—	—	—	—	—	—	—	—	—
25—29	2	226 5	91 0	66 5	2 27	1 3	1 23	0 279	0 371	0 263
30—34	1	225 0	—	—	1 84	—	—	0 264	—	—
35—39	15	270 9	62 8	16 2	1 72	1 01	0 2	0 176	0 228	0 059
40—44	38	289 7	68 1	11 0	2 02	1 04	0 17	0 247	0 230	0 037
45—49	32	290 9	51 9	9 2	1 96	0 85	0 15	0 254	0 180	0 032
50 54	25	292 7	56 2	11 2	1 87	0 81	0 16	0 227	0 210	0 012
55—59	15	282 3	54 2	14 0	2 10	0 89	0 23	0 281	0 201	0 052
60 64	6	290 3	68 9	28 1	1 53	0 66	0 27	0 150	0 194	0 080
65 69	3	261 7	47 5	27 4	1 34	0 25	0 15	0 120	0 084	0 049
70 74	0	—	—	—	—	—	—	—	—	—

N number of subjects $\bar{x}$ mean value s = standard de at on s — s andard error of the mean

TABLE N 22 Concen ra on of choles erol and tr glycer des and log concentrat on of tr glycer des for women in group 4

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	x mg/100 ml	s mg/100 ml	s_x mg/100 ml	x mmole/l	s mmole/l	s_x mmole/l	x log mmole/l	s log mmole/l	s_x log mmole/l
15 19	0							—	—	—
20 24	3	[illegible] 0	45 9	26 5	1 21	0 55	0 3	0 055	0 18	0 10
25 29	0		—		—	—	—			
30 34	0				—				—	—
35 39		40	42 3	1 3	1 16	0 50	0 20	0 0 5	0 20	0 081
40 44	19	7 1	38 6	8 9	1 09	0 31	0 0	0 021	0 113	0 0 6
45 49		87 1	41 6	11 9	1 34	0 38	0 07	0 109	0 123	0 0 4
50 54	18	81 4	49 6	11 7	1 12	0 42	0 10	0 0 1	0 160	0 038
55 59	11	34 3	63 1	19 0	1 9	0 44	0 19	0 1 0	0 1 3	0 0
60 64	8	31 1	8	30	1 48	0 52	0 19	0 146	0 15	0 05
65 69	4	3 3	0	3 4	1 85	0 94	0 4	0 231	0 193	0 09
70 74										

N = number of subjects x mean value s = standard deviation s_x = standard error of the mean

Table A 23 Concentration of cholesterol and triglycerides and log concentration of triglycerides for men in group 5

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	$s_{\bar{x}}$ mg/100 ml	$\bar{x}$ mmole/l	s mmole/l	$s_{\bar{x}}$ mmole/l	$\bar{x}$ log mmole/l	s log mmole/l	$s_{\bar{x}}$ log mmole/l
15—19	0	—	—	—	—	—	—	—	—	—
20—24	0	—	—	—	—	—	—	—	—	—
25—29	0	—	—	—	—	—	—	—	—	—
30—34	1	274.0	—	—	2.76	—	—	0.440	—	—
35—39	0	—	—	—	—	—	—	—	—	—
40—44	0	—	—	—	—	—	—	—	—	—
45—49	3	329.7	54.8	31.6	2.60	1.28	0.74	0.380	0.208	0.120
50—54	4	259.5	45.9	22.9	1.05	0.41	0.20	—0.002	0.165	0.083
55—59	11	286.1	65.6	19.8	2.11	2.02	0.61	0.193	0.343	0.104
60—64	2	363.0	77.8	55.0	1.54	0.27	0.19	0.184	0.076	0.054
65—69	2	333.5	16.3	11.5	1.84	0.35	0.25	0.259	0.082	0.058
70—74	1	316.0	—	—	0.74	—	—	—0.131	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

Table A 24 Concentration of cholesterol and triglycerides and log concentration of triglycerides for women in group 5

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	$s_{\bar{x}}$ mg/100 ml	$\bar{x}$ mmole/l	s mmole/l	$s_{\bar{x}}$ mmole/l	$\bar{x}$ log mmole/l	s log mmole/l	$s_{\bar{x}}$ log mmole/l
15—19	0	—	—	—		—	—	—	—	—
20—24	0	—	—	—	—	—	—	—	—	—
25—29	0	—	—	—	—	—		—	—	—
30—34	0	—	—	—	—	—	—	—	—	—
35—39	0	—	—	—	—	—	—	—	—	—
40—44	2	236.0	65.1	46.0	1.41	0.39	0.28	0.139	0.121	0.086
45—49	0	—	—	—	—		—	—	—	—
50—54	2	250.0	14.1	10.0	2.47	1.35	0.96	0.356	0.251	0.178
55—59	0		—	—	—	—	—	—	—	—
60—64	1	333.0	—	—	1.36	—	—	0.133	—	—
65—69	0	—		—	—			—	—	—
70—74	0	—		—	—	—	—	—	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

TABLE A 25 Concentration of cholesterol and triglycerides and log concentration of triglycerides for men in group 6

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	$s_{\bar{x}}$ mg/100 ml	$\bar{x}$ mmole/l	s mmole/l	$s_{\bar{x}}$ mmole/l	$\bar{x}$ log mmole/l	s log mmole/l	s log mmole/l
15—19	0	—	—	—	—	—	—	—	—	—
20—24	0	—	—	—	—	—	—	—	—	—
25—29	0	—	—	—	—	—	—	—	—	—
30—34	0	—	—	—	—	—	—	—	—	—
35—39	0	—	—	—	—	—	—	—	—	—
40—44	4	252 0	27 5	13 8	1 42	1 07	0 54	0 073	0 292	0 146
45—49	4	282 0	73 5	36 8	1 47	0 52	0 26	0 148	0 138	0 069
50—54	7	313 0	79 3	30 0	1 56	0 77	0 29	0 159	0 172	0 065
55—59	4	244 8	98 3	49 2	1 59	0 91	0 45	0 152	0 227	0 114
60—64	5	275 2	17 9	8 0	1 54	0 85	0 38	0 140	0 218	0 093
65—69	1	326 0	—	—	1 52	—	—	0 181	—	—
70—74	0	—	—	—	—	—	—	—	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

TABLE A 26 Concentration of cholesterol and triglycerides and log concentration of triglycerides for women in group 6

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	s mg/100 ml	$\bar{x}$ mmole/l	s mmole/l	s mmole/l	$\bar{x}$ log mmole/l	s log mmole/l	s log mmole/l
15—19	0		—	—	—	—	—	—	—	—
20—24	0				—	—	—	—	—	—
25—29	0				—	—	—	—	—	—
30—34	1	258 0		—	0 56	—		—0 25[illegible]	—	—
35—39	1	[illegible]70 0			0 70	—	—	—0 155	—	
40—44	[illegible]	[illegible]	74 9	30 6	1 22	0 28	0 11	0 074	0 101	0 041
45—49	4	[illegible]80 0	88 7	44 3	0 94	0 09	0 05	—0 030	0 044	0 022
50—54	3	303	12 0	6 9	1 08	0 18	0 10	0 030	0 070	0 041
55—59	4	[illegible]	48 2	24 1	1 27	0 18	0 09	0 101	0 056	0 078
60—64	3	[illegible]	31 [illegible]	18 0	1 84	0 31	0 18	0 260	0 074	0 043
65—69		303 0	35 4	25 0	1 03	0 04	0 03	0 031	0 014	0 010
70—74	0							—	—	—

N number of subjects x mean value s standard deviation s standard error of the mean

TABLE A 27 Concentration of cholesterol and triglycerides and log concentration of triglycerides for men in group 8

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	$s_{\bar{x}}$ mg/100 ml	$\bar{x}$ mmole/l	s mmole/l	$s_{\bar{x}}$ mmole/l	$\bar{x}$ log mmole/l	s log mmole/l	$s_{\bar{x}}$ log mmole/l
15—19	0	—	—	—	—	—	—	—	—	—
20—24	0	—	—	—	—	—	—	—	—	—
25—29	3	216.0	105.4	60.9	1.22	0.63	0.36	0.011	0.247	0.143
30—34	4	249.5	34.8	17.4	1.19	0.89	0.44	0.000	0.2[illegible]4	0.137
35—39	9	280.0	59.2	19.7	1.50	0.53	0.18	0.152	0.148	0.049
40—44	19	266.1	48.7	11.2	1.54	0.65	0.15	0.151	0.182	0.04[illegible]
45—49	17	272.6	62.5	15.2	1.54	0.81	0.20	0.140	0.200	0.049
50—54	9	279.7	45.9	15.3	1.56	1.01	0.34	0.136	0.219	0.0[illegible]3
55—59	10	287.9	29.7	9.4	1.59	0.55	0.17	0.176	0.149	0.04[illegible]
60—64	4	297.8	69.0	34.5	1.53	0.64	0.32	0.154	0.188	0.094
65—69	1	275.0	—	—	1.51	—	—	0.1[illegible]9	—	—
70—74	0	—	—	—	—	—	—	—	—	

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ standard error of the mean

TABLE A 28 Concentration of cholesterol and triglycerides and log concentration of triglycerides for women in group 8

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	$s_{\bar{x}}$ mg/100 ml	$\bar{x}$ mmole/l	s mmole/l	$s_{\bar{x}}$ mmole/l	$\bar{x}$ log mmole/l	s log mmole/l	$s_{\bar{x}}$ log mmole/l
15—19	0	—	—	—	—	—	—	—	—	—
20—24	0	—	—	—	—		—	—	—	—
25—29	6	260.5	31.2	12.8	1.08	0.38	0.15	0.011	0.157	0.064
30—34	4	249.0	31.4	15.7	1.16	0.59	0.29	0.019	0.234	0.117
35—39	10	252.4	50.7	16.0	0.90	0.36	0.12	−0.076	0.1[illegible]5	0.055
40—44	15	291.5	6.0	19.6	1.45	0.74	0.19	0.110	0.272	0.057
45—49	5	298.0	31.4	14.1	1.30	0.45	0.20	0.085	0.185	0.083
50—54	13	294.8	41.0	11.4	1.34	0.59	0.16	0.100	0.151	0.04[illegible]
55—59	4	290.3	38.3	19.2	0.99	0.53	0.27	−0.067	0.291	0.146
60—64	1	340.0	—	—	1.12	—	—	0.049	—	—
65—69	0	—	—	—	—	—	—		—	—
70—74	0	—	—	—	—	—	—	—	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

TABLE A 29 Concentration of cholesterol and triglycerides and log concentration of triglycerides for men in group 9

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	s_x mg/100 ml	$\bar{x}$ mmole/l	s mmole/l	s_x mmole/l	$\bar{x}$ log mmole/l	s log mmole/l	s_x log mmole/l
15—19	0	—	—	—	—	—	—	—	—	—
20—24	2	273 5	0 0	49 5	0 81	0 01	0 01	—0 092	0 007	0 00
25—29	10	244	74 1	3 4	1 60	0 76	0 24	0 157	0 2 1	0 0 0
30—34	4	260 5	51 3	20 9	1 45	0 26	0 11	0 153	0 083	0 034
35—39	21	261 8	0 9	11 1	1 60	0 73	0 16	0 155	0 212	0 046
40—44	32	278 3	60 3	10 7	1 47	0 84	0 15	0 116	0 202	0 03
45—49	24	287 4	45 9	9 4	1 43	0 46	0 09	0 134	0 141	0 029
50—54	18	9 3	50 0	11 8	1 36	0 55	0 13	0 100	0 173	0 041
55—59	9	287 6	40 4	13 5	1 83	0 92	0 31	0 221	0 194	0 065
60—64	4	294 8	16 3	6 7	1 39	0 50	0 21	0 123	0 137	0 06
65—69	1	94 0	—	—	1 4	—	—	0 161	—	—
70—74	0	—	—	—	—	—	—	—	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation s_x = standard error of the mean

TABLE A 30 Concentration of cholesterol and triglycerides and log concentration of triglycerides for women in group 9

Age years	Cholesterol				Triglycerides			log Triglycerides		
	N	$\bar{x}$ mg/100 ml	s mg/100 ml	s_x mg/100 ml	$\bar{x}$ mmole/l	s mmole/l	s_x mmole/l	$\bar{x}$ log mmole/l	s log mmole/l	s_x log mmole/l
15—19	0				—	—	—		—	—
20—24	1	199 0	—		0 54		—	—0 2 8		
25—29	3	21 7	31 0	1 9	0 87	0	0 30	—0 119	0 291	0 10
30—34	8	97 8	1 0 3	1 5	1 22	0 60	0 21	0 042	0 210	0 0 4
35—39			1 3	10 9	1 2	0 62	0 13	0 057	0 193	0 041
40—44		3	49 4	9 9	1 1	0 7	0 11	0 017	0 1 3	0 03
45—49	18		9	13 4	1 10	0 0	0 1	0 009	0 1	0 03
50—54	4		81 3	20 5	1	1 43	0 5	0 112	0 30	0 0
55—59	4		41 3	14	1 4	0 58	0 20	0 131	0 193	0 068
60—64	5	3 8	1	3 1	1	1 0	0 1	0 243	0 219	0 12
65—69									—	—
70—74									—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation s_x = standard error of the mean

TABLE A 31 Systolic and diastolic blood pressure for men and index of skewness of distribution of values within each age class of group 10

Age years	Systolic blood pressure						Diastolic blood pressure				
	N	$\bar{x}$ mm Hg	s mm Hg	$s_{\bar{x}}$ mm Hg	g	s_g	$\bar{x}$ mm Hg	s mm Hg	$s_{\bar{x}}$ mm Hg	g	s_g
15—19	3	133.3	15.3	8.8	0.94	1.22	86	5.8	3.3	1.73	1.22
20—24	15	129.7	11.3	2.9	0.18	0.58	80	6.5	1.7	0.39	0.58
25—29	33	130.2	8.9	1.5	0.52	0.41	78.9	6.7	1.2	0.25	0.41
30—34	71	129.9	11.9	1.4	0.74**	0.28	81.5	7.3	0.9	0.36	0.28
35—39	179	131.1	12.4	0.9	0.59**	0.18	83.7	8.4	0.6	0.1	0.18
40—44	289	134.3	13.6	0.8	0.44**	0.14	84.7	8.0	0.5	—0.03	0.14
45—49	267	135.9	13.4	0.8	0.3 *	0.15	86.2	8.0	0.5	0.05	0.15
50—54	202	137.2	14.6	1.0	0.16	0.17	86.5	8.0	0.6	—0.10	0.1
55—59	126	140.3	13.6	1.2	0.50*	0.22	87.2	7	0.7	—0.03	0.22
60—64	55	142.5	15.1	2.0	0.20	0.32	86.6	7.8	1.0	—0.27	0.32
65—69	21	138.8	13.1	2.9	0.27	0.50	81.8	7.8	1	—0.26	0.50
70—74	5	163.0	7.6	3.4	—1.75	0.91	92.0	5.7	2.5	0.40	0.91

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean g = index of skewness s_g = standard error of g

TABLE A 32 Systolic and diastolic blood pressure for women and index of skewness of distribution of values within each age class of group 10

Age years	Systolic blood pressure						Diastolic blood pressure				
	N	$\bar{x}$ mm Hg	s mm Hg	$s_{\bar{x}}$ mm Hg	g	s_g	$\bar{x}$ mm Hg	s mm Hg	$s_{\bar{x}}$ mm Hg	g	s_g
15—19	8	120.0	5.3	1.9	0.94	0.75	76.3	4.4	1.6	0.62	0.75
20—24	39	119.7	6.6	1.1	—1.00**	0.38	75.3	6.7	1.1	—1.21	0.38
25—29	37	122.0	12.2	2.0	1.41***	0.39	77.2	8.8	1.4	0.59	0.39
30—34	32	121.3	9.7	1.7	0.05	0.41	77.0	6.5	1.1	0.32	0.41
35—39	112	127.9	12.8	1.2	0.61**	0.23	81.4	7.9	0.7	0.68**	0.23
40—44	259	130.1	13.7	0.8	0.70***	0.15	82.2	8.1	0.5	0.28	0.15
45—49	208	132.7	13.5	0.9	0.19	0.17	82.6	8.0	0.6	—0.01	0.17
50—54	152	138.8	14.8	1.2	0.45*	0.20	85.3	8.5	0.7	0.02	0.20
55—59	69	145.0	14.6	1.8	—0.06	0.29	89.1	7.5	0.9	—0.31	0.29
60—64	18	151.0	17.9	4.2	—0.61	0.54	90.8	6.5	1.5	—0.53	0.54
65—69	4	148.8	13.1	6.6	—0.12	1.01	88.8	6.3	3.1	—1.13	1.01
70—74	1	170.0	—	—	—		90.0	—	—	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean g = index of skewness s_g = standard error of g

TABLE A 33 The regressions of systolic and diastolic blood pressure (mm Hg) on age (years) for men in group 10

Age years	Systolic blood pressure					Diastolic blood pressure			
	N	b	s	s_b	t	b	s	s_b	t
15—19	3	—20 00	14 14	17 32	—1 154	—10 00	0 00	0 00	—
15—24	18	—1 22	11 53	1 17	—1 047	—0 78	6 57	0 66	—1 180
15—29	51	—0 17	9 88	0 47	—0 359	—0 26	6 77	0 32	—0 802
15—34	122	—0 02	11 10	0 24	—0 070	0 19	7 11	0 16	1 225
15—39	301	0 10	11 86	0 14	0 691	0 30**	7 92	0 09	3 186
15—44	590	0 32***	12 75	0 10	3 294	0 29***	7 95	0 06	4 729
15—49	857	0 35***	12 93	0 07	4 961	0 28***	7 95	0 04	6 500
15—54	1059	0 35***	13 26	0 06	6 040	0 25***	7 95	0 03	7 216
15—59	1185	0 37***	13 30	0 05	7 717	0 23***	7 93	0 03	7 943
15—64	1240	0 38***	13 38	0 04	8 679	0 21***	7 93	0 03	7 808
15—69	1261	0 37***	13 38	0 04	8 644	0 18***	7 95	0 03	7 348
15—74	1266	0 39***	13 41	0 04	9 308	0 19***	7 94	0 02	7 578
15—79	1266	0 39***	13 41	0 04	9 308	0 19***	7 94	0 02	7 578
20—79	1263	0 40***	13 40	0 04	9 384	0 19***	7 94	0 02	7 722
25—79	1248	0 41***	13 42	0 04	9 290	0 19***	7 95	0 03	7 382
30—79	1215	0 43***	13 2	0 05	9 003	0 17***	7 98	0 03	6 049
35—79	1144	0 43***	13 62	0 05	8 163	0 15***	8 01	0 03	4 638
40—79	965	0 41***	13 84	0 07	6 184	0 12**	7 93	0 04	3 276
45—79	676	0 46***	13 96	0 10	4 849	0 06	7 89	0 05	1 125
50—79	409	0 53***	14 38	0 15	3 542	0 04	7 84	0 08	0 489
55—79	207	0 50*	14 17	0 25	2 011	—0 00	7 74	0 14	—0 021
60—79	81	0 73	15 08	0 52	1 387	0 03	7 81	0 27	0 104
65—79	26	3 36**	13 48	1 11	3 037	0 73	7 87	0 65	1 126
70—79	5	—3 37	6 47	2 13	—1 579	—1 52	6 02	1 98	—0 766

N number of subjects b = regression coefficient s = standard deviation from regression s_b = standard deviation of the regression coefficient t = b/s_b

TABLE A 34 *The regressions of systolic and diastolic blood pressure (mm Hg) on age (years) for women in group 10*

Age years	Systolic blood pressure					Diastolic blood pressure			
	N	b	s	s_b	t	b	s	s_b	t
15—19	8	0 00	5 77	1 67	0 000	1 25	4 45	1 28	0 973
15—24	47	0 10	6 40	0 45	0 218	—0 15	6 39	0 45	—0 328
15—29	84	0 39	9 32	0 30	1 315	0 22	7 57	0 24	0 918
15—34	116	0 21	9 39	0 18	1 158	0 17	7 21	0 14	1 169
15—39	278	0 52***	11 25	0 11	4 582	0 37***	7 58	0 08	4 876
15—44	497	0 55***	12 59	0 08	6 605	0 34***	7 85	0 05	6 605
15—49	705	0 53***	12 88	0 07	7 946	0 28***	7 90	0 04	6 888
15—54	857	0 62***	13 28	0 06	10 986	0 30***	8 01	0 03	8 786
15—59	926	0 69***	13 47	0 05	13 521	0 34***	7 99	0 03	11 093
15—64	944	0 72***	13 54	0 05	14 560	0 35***	7 97	0 03	11 908
15—69	948	0 72***	13 53	0 05	14 767	0 35***	7 96	0 03	12 014
15—74	949	0 73***	13 54	0 05	14 957	0 35***	7 96	0 03	12 111
15—79	949	0 73***	13 54	0 05	14 957	0 35***	7 96	0 03	12 111
20—79	941	0 75***	13 57	0 05	14 764	0 35***	7 98	0 03	11 887
25—79	902	0 81***	13 77	0 06	13 469	0 36***	8 03	0 03	10 211
30—79	865	0 86***	13 82	0 07	12 580	0 36***	8 00	0 04	9 147
35—79	833	0 85***	13 96	0 08	11 379	0 34***	8 06	0 04	7 935
40—79	721	0 93***	14 11	0 09	10 312	0 39***	8 08	0 05	7 436
45—79	452	1 10***	14 37	0 14	7 665	0 52***	8 04	0 08	6 432
50—79	244	1 05***	14 93	0 24	4 304	0 50***	8 05	0 13	3 814
55—79	92	0 92	15 16	0 49	1 894	0 26	7 22	0 23	1 112
60—79	23	0 62	17 26	1 31	0 469	0 03	6 42	0 49	0 066
65—79	5	2 50	15 94	3 68	0 679	1 60	5 83	1 35	1 185

N = number of subjects b = regression coefficient s = standard deviation from regression s_b = standard deviation of the regression coefficient $t = b/s_b$

Table A 35 Systolic and diastolic blood pressure for men and women in group 1

Age years	Men							Women						
	N	Systolic blood pressure mm Hg			Diastolic blood pressure mm Hg			N	Systolic blood pressure mm Hg			Diastolic blood pressure mm Hg		
		$\bar{x}$	s	s	$\bar{x}$	s	s_x		$\bar{x}$	s	s	$\bar{x}$	s	$s_{\bar{x}}$
15 19	2	127 5	10 6	7 5	77 5	3 5	2 5	6	121 7	12 1	4 9	75 8	6 6	2 7
20 24	7	125 0	9 1	3 5	72 9	4 9	1 8	20	116 3	8 6	1 9	69 3	6 7	1 5
25 29	17	126 8	10 3	2 5	79 1	5 9	1 4	27	125 9	17 9	3 4	75 0	10 8	2 1
30 34	0	129 9	13 9	2 0	82 8	9 4	1 3	50	123 0	8 7	1 2	78 0	7 4	1 0
35 39	124	133 6	12 1	1 1	84 9	9 0	0 8	133	127 0	13 1	1 1	80 9	9 1	0 8
40 44	311	135 3	16 3	0 9	85 2	9 1	0 5	339	131 6	19 1	1 0	82 6	10 3	0 6
45 49	316	139 6	16 5	0 9	87 8	10 3	0 6	302	137 7	17 7	1 0	85 4	9 8	0 6
50 54	335	143 8	18 6	1 0	89 3	11 4	0 6	268	146 3	22 8	1 4	89 5	11 0	0 7
55 59	234	151 8	23 1	1 3	92 5	11 0	0 8	216	150 3	23 2	1 6	89 8	12 9	0 9
60 64	194	150 0	21 4	1 5	91 9	11 2	0 8	96	162 0	23 7	2 4	93 1	12 8	1 3
65 69	85	155 1	18 7	2 0	93 2	10 1	1 1	27	171 1	24 5	4 7	97 0	12 3	2 4
70 74	20	174 5	27 8	6 2	100 5	13 5	3 0	3	168 3	46 5	26 8	96 7	11 5	6 7
75 79	2	167 5	31 8	22 5	100 0	35 4	25 0	0	—	—	—	—	—	—

N number of subjects $\bar{x}$ = mean value s = standard deviation s_x = standard error of the mean

TABLE A 36 Syst l c a l d a t l c blo d pres ure for en a l wo ne in gro p 2

Age years	Men N	Sys l c blo l p e sure Hg x	s	s_x	D ast l bl l press re n Hg x	s	s_x	Wo e N	Systol l l press re n Hg x	s	s_x	D a tol c l lo l pressure Hg x	s	s_x
l 19	0							0						
0 21	0							0						
25 29	0							0						
30 34	2	14 0	7 1	5 0	142 5	3 0	3	(						
3 39	3	1(8 3	7	4 4	113 3	8	3 3	4	173 8	11 1		11 5	12 (	9
40 44	27	172	1(7	3 2	112 8	8)	1 7	13	177 7	14 8	1 1	1(0 8	1	1 7
45 49	2(	177 7	1J 1	3 7	113 1	11 0	2 1	11	187)	18	1)	1(8)	) 8	(
0 54	27	170 4	1( )	3 3	110	7	1	18	177	14 1	1 1	104 1	10	2
5—59	20	18 3	1 1	4 3	111 8	11 4			1) 1	21 (	1 1	111)	1	3 4
(0 (4	23	1J1 3	15 8	3 3	110 0	) 0	1)	7	18 7	1 7	1 8	101 3	1	3
(—( )		0?0	1J(	8 7	111 0	11 4	1		2(0			10 0	1	(
70—74	3	181 7	7	4 4	103 3	11 5	7	1	180 0			11 0		

N n l er f aul je ts x e l s st l d le a s s d r l er r l l e e

TABLE A 37 Systolic and diastolic blood pressure for men and women in group 3

Age years	Men							Women						
	N	Systolic blood pressure mm Hg			Diastolic blood pressure mm Hg			N	Systolic blood pressure mm Hg			Diastolic blood pressure mm Hg		
		$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$		$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	s
15 19	0		—	—	—	—	—	0	—	—	—	—	—	—
20 24	0					—	—	2	135.0	28.3	20.0	85.0	14.1	10.0
25 29	0			—	—		—	0	—	—	—	—	—	—
30 34	1	120.0			75.0	—	—	1	115.0	—	—	70.0	—	—
35 39	1	155.0	—		95.0	—	—	1	145.0	—	—	95.0	—	—
40 44	5	1 0.0	14.6	6.5	89.0	8.2	3.7	5	136.0	19.8	8.9	88.0	10.4	4.6
45 49	10	142.5	12.5	4.0	89.5	6.9	2.2	8	131.4	14.0	4.9	83.8	7.9	2.8
50 54	9	146.7	13.7	4.6	88.3	7.5	2.5	6	139.2	11.1	4.5	89.2	3.8	1.5
55 59	8	154.4	11.2	3.9	92.?	6.5	2.3	4	160.0	20.0	10.0	97.?	5.0	2.5
60 64	5	166.0	5.5	2.4	95.0	5.0	2.2	3	168.3	2.9	1.7	93.3	11.?	6.7
65 69	2	15?.?	24.7	17.5	85.0	7.1	5.0	1	150.0	—	—	80.0	—	—
70 74	2	160.0	14.1	10.0	95.0	7.1	5.0	0	—	—	—	—	—	—

N number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

TABLE A 38 Systolic and diastolic blood pressure for men and women in group 4

Age years	Men N	Systolic blood pressure mm Hg			Diastolic blood pressure mm Hg			Women N	Systolic blood pressure mm Hg			Diastolic blood pressure mm Hg		
		$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$		$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$
15–19	0	—						0						
20–24	0							3	138.3	12.6	7.3	86.7	10.4	6.0
25–29	2	110.0			87	10.4	7	0						
30–34	1	130.0			85.0			0						
35–39	15	132.3	10.0	2.6	84.0	[illegible]	[illegible]	[illegible]	132	[illegible]	8.3	88.3	11.3	[illegible]
40–44	30	140.7	13.1	2.1	88.3	[illegible]	1.0	19	131.3	8.3	[illegible]	83.7	[illegible]	[illegible]
45–49	32	141.9	14.9	[illegible]	89.2	8.4	[illegible]	27	135	10.3	[illegible]	87.[illegible]	[illegible]	[illegible]
50–54	25	147.4	14.2	2.8	91.0	7.6	[illegible]	18	[illegible]	12.0	2.8	8[illegible].3	7.4	1.7
55–59	15	149.3	13.7	3.5	83.0	7.1	1.8	11	112.3	[illegible]	[illegible]	8[illegible].8	7.8	[illegible]
60–64	6	145.8	21.5	8.8	91.7	[illegible]	[illegible]	8	118.8	11.3	[illegible]	88.1	[illegible]	[illegible]
65–69	3	150.0	15.0	8.6	95.0	[illegible]	[illegible]	4	[illegible]	10.8	[illegible]	91.3	[illegible]	3.1
70–74	0							0						

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

Table V 41 Systolic and diastolic blood pressure for men and women in group B

Age years	Men							Women						
	N	Systolic blood pressure mm Hg			Diastolic blood pressure mm Hg			N	Systolic blood pressure mm Hg			Diastolic blood pressure mm Hg		
		$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$		$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$
15 19	0							0	—	—		—	—	
20 24	0							0	—	—		—		—
25 29	3	[illegible]	[illegible]	1 7	73 3	[illegible]	1 7	[illegible]	[illegible]	11 7	4 8	81 7	8 2	3 3
30 34	4	[illegible]	7 5	3 8	81 3	[illegible]	3 1	4	131 3	11 1	[illegible]	[illegible]	[illegible]	3 8
35 39	[illegible]	[illegible]	[illegible]	[illegible]	85 0	9 7	3 2	10	122 0	10 3	3 3	81 0	[illegible]	1 8
40 44	[illegible]	132 9	[illegible]	[illegible]	85 5	9 1	2 1	[illegible]	[illegible]	[illegible]	3 2	79 0	[illegible]	2 0
45 49	17	133 8	10 7	[illegible]	83 2	[illegible]	1 4	[illegible]	132 0	11 5	[illegible]	84 0	[illegible]	2 4
50 54	9	[illegible]	8 6	[illegible]	[illegible]	7 7	[illegible]	13	133 1	13 5	3 7	84 2	4 5	1 2
55 59	10	141 5	9 7	3 1	90 0	[illegible]	1 8	1	111 3	13 2	[illegible]	91 3	[illegible]	3 1
60 64	4	137 5	[illegible]	[illegible]	[illegible]	[illegible]	2 9	1	145 0	—	—	90 0	—	—
65 69	1	[illegible]			90 0	—	—	0	—	—	—	—	—	—
70 74	0	—			—	—	—	0	—	—	—	—	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

TABLE A 42 Systolic and diastolic blood pressure for men and women in group 9

Age years	Men							Women						
	N	Systolic blood pressure mm Hg			Diastolic blood pressure mm Hg			N	Systolic blood pressure mm Hg			Diastolic blood pressure mm Hg		
		$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$		$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$
15—19	0							0	—					
20—24	[illegible]	117.5	10.6	[illegible]	[illegible] 0.0	—	—	1	115.0	—		90.0		
25—29	10	135.[illegible]	14.4	4.6	83.0	8.6	2.7	3	120.0	10.0	5.8	[illegible]7	5.8	3.3
30—34	6	135.0	16.1	6.[illegible]	86	10.3	4.2	8	120.6	[illegible]	2.0	100.0	[illegible]0	2.1
35—39	21	13[illegible].1	11.[illegible]	2.[illegible]	8[illegible]	[illegible]8	1.7	22	12[illegible].4	11[illegible]	[illegible]	[illegible]8	10.3	2.2
40—44	32	133.8	13.2	2.3	84	4	1.3	25	13[illegible]	10[illegible]	1	[illegible]4	[illegible]	1.4
45—49	4	138.8	13.0	[illegible]	81.[illegible]	18.[illegible]	3.8	18	133.3	[illegible]	[illegible].5	[illegible]	10.0	[illegible].4
50—54	19	13[illegible].1	13.6	3.[illegible]	88.[illegible]	8.5	2.0	1	14[illegible].1	[illegible]	3	[illegible]4	7	1.[illegible]
55—59	9	141.1	12	4.[illegible]	8[illegible]	[illegible]0	1	8	[illegible]3	[illegible]	[illegible]	1	4[illegible]	1.[illegible]
60—64	6	138.0	1[illegible].9	[illegible]3	8[illegible].9	8.0	3.3	[illegible]	[illegible]	[illegible]	3			4.[illegible]
65—69	1	170.0			90.0	—		0	[illegible]					
70—74	0							1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

N = number of subjects, $\bar{x}$ = mean value, s = standard deviation, $s_{\bar{x}}$ = standard [illegible]

TABLE A 43 Weight-height index kg (cm − 100) and index of skewness of distribution of values for men and women in group 10

Age year	Men						Women					
	N	$\bar{x}$ kg/(cm − 100)	s kg/(cm − 100)	$s_{\bar{x}}$ kg/(cm − 100)	g	s_g	N	$\bar{x}$ kg/(cm − 100)	s kg/(cm − 100)	$s_{\bar{x}}$ kg/(cm − 100)	g	s_g
15–19	3	0.810	0.015	0.026	0.91	1.22	8	0.860	0.153	0.054	0.01	0.75
20–24	15	0.877	0.093	0.024	0.09	0.58	39	0.847	0.080	0.013	0.48	0.38
25–29	33	0.880	0.096	0.015	−0.11	0.41	37	0.877	0.114	0.019	0.33	0.39
30–34	71	0.906	0.090	0.011	−0.12	0.28	42	0.927	0.089	0.016	0.26	0.41
35–39	179	0.921	0.103	0.008	−2.19***	0.18	112	0.924	0.096	0.009	0.54**	0.23
40–44	83	0.911	0.106	0.006	−2.25***	0.14	269	0.936	0.093	0.006	0.29	0.15
45–49	267	0.930	0.086	0.005	0.21	0.15	208	0.941	0.109	0.008	−2.57***	0.17
50–54	202	0.912	0.109	0.008	−2.00***	0.17	152	0.958	0.099	0.008	0.12	0.20
55–59	126	0.948	0.116	0.010	−3.38***	0.22	63	0.963	0.092	0.011	−0.05	0.29
60–64	55	0.948	0.112	0.015	−0.29	0.32	18	0.998	0.114	0.027	0.78	0.54
65–69	21	0.924	0.120	0.026	0.70	0.50	4	0.967	0.163	0.085	1.03	1.01
70–74	5	0.912	0.016	0.021	−1.18	0.91	1	1.030	0.000	0.000	0.00	0.00

N = number of subjects; $\bar{x}$ = mean value; s = standard deviation; $s_{\bar{x}}$ = standard error of the mean; g = index of skewness; s_g = standard error of g

TABLE A 44 The regression of weight height index kg cm—100 on age years for men and women in group 10

Age years	Men					Women				
	N	b	s	s_b	t	N	b	s	s_b	t
15—19	3	—0 074	0 021	0 0759	—2 886	8	0 000	0 1 6	0 04 9	0 11
15—24	18	0 014	0 085	0 0086	1 640	4	—0 003	[illegible]	[illegible]	0
15—29	51	0 005	0 086	0 0040	1 2 2	64	0 013	[illegible]	013	1 0
15—34	122	0 004*	0 088	0 0019	2 059	116	0 00 *		017	3 91
15—39	301	0 003**	0 100	0 0011	3 323	278	0 004***	[illegible]	0 09	4
15—44	590	0 003***	0 103	0 0007	5 039	49	0 003 **	0 09	[illegible]	[illegible]
15—49	837	0 003***	0 098	0 0005	6 343	05	0 003***	[illegible]		[illegible]
15—54	1059	0 002***	0 100	0 0004	5 577	85	0 003 **		04	[illegible]
15—59	1185	0 002***	0 102	0 0003	5 396	9 6	0 00 ***	[illegible]	0 3	[illegible]
15—64	1240	0 001***	0 103	0 0003	5 2 3	944	0 0 ***		[illegible]	
15—69	1261	0 001***	0 103	0 0003	4 789	948	0 002***	[illegible]	[illegible]	[illegible]
15—74	1266	0 001***	0 103	0 0003	4 671	949	0 002***	[illegible]	[illegible]	8 1
15— 9	1 66	0 001***	0 103	0 0003	4 671	949	0 002***	0 9	000	8
20— 9	1263	0 001***	0 103	0 0003	4 350	941	0 00 ***	0 0 9	0 1	
2 —79	1248	0 001***	0 103	0 0003	3 859	904	0 002***	0 100	0 0 4	9
30—79	1215	0 001**	0 104	0 0003	2 873	8 5	0 00 ***	0 099	0 0 04	4 40
35— 9	1144	0 000	0 104	0 0004	1 881	833	0 007***	0 099	0 000	4 138
40—79	965	0 000	0 103	0 0004	0 1 9	721	0 007***	0 100	0 000	3 4 8
45—79	676	—0 000	0 107	0 000	-0 13	452	0 003**	0 103	0 0010	2 5
50— 9	409	—0 000	0 112	0 0011	—0 247	244	0 002	0 098	0 001	1 9
55—79	207	—0 001	0 114	0 0070	—0 740	92	0 004	0 100	0 0032	1 24
60— 9	81	—0 004	0 111	0 0038	— 1 071	23	0 006	0 170	0 0091	0 687
6 — 9	26	—0 000	0 111	0 0091	—0 0 9	5	0 0 0	0 119	0 0	1 81
70— 9	5	0 008	0 051	0 0170	0 483	0		—		

N = number of subjects b = regression coefficient s standard deviation from regression s_b standard deviation of the regression coefficient t b s_b

TABLE A 45 Weight height index kg/(cm—100) for men and women in group 1

Age years	Men				Women			
	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg/(cm—100)	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg/(cm—100)
15—19	2	0.830	0.169	0.1199	6	0.828	0.109	0.0448
20—24	7	0.772	0.081	0.0309	20	0.884	0.161	0.0360
25—29	17	0.884	0.092	0.0223	27	0.897	0.140	0.0270
30—34	50	0.930	0.096	0.0136	50	0.917	0.107	0.0151
35—39	124	0.936	0.110	0.0099	133	0.937	0.127	0.0110
40—44	311	0.957	0.118	0.0067	339	0.969	0.130	0.0071
45—49	316	0.979	0.179	0.0096	302	0.992	0.143	0.0082
50—54	335	0.982	0.120	0.0065	268	1.022	0.148	0.0090
55—59	294	0.984	0.109	0.0064	216	1.078	0.160	0.0109
60—64	194	0.987	0.132	0.0095	96	1.040	0.153	0.0156
65—69	85	0.953	0.117	0.0127	27	1.031	0.134	0.0258
70—74	20	1.014	0.120	0.0269	3	0.983	0.304	0.1757
75—79	2	1.145	0.162	0.1150	0	—	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

TABLE A 46 Weight height index kg/(cm—100) for men and women in group 2

Age years	Men				Women			
	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg/(cm—100)	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg/(cm—100)
15—19	0		—	—	0	—	—	—
20—24	0	—	—	—	0	—	—	—
25—29	0	—		—	0	—	—	—
30—34	2	0.815	0.148	0.1050	0	—	—	—
35—39	3	1.066	0.219	0.1267	4	0.950	0.086	0.0430
40—44	27	1.018	0.124	0.0240	13	1.084	0.196	0.0546
45—49	26	1.029	0.109	0.0214	14	1.039	0.157	0.0421
50—54	27	0.995	0.112	0.0216	18	1.060	0.111	0.0262
55—59	20	1.015	0.128	0.0288	26	1.111	0.152	0.0299
60—64	23	1.035	0.144	0.0300	7	1.030	0.163	0.0616
65—69	5	1.122	0.100	0.0448	2	0.955	0.021	0.0149
70—74	3	0.990	0.200	0.1159	1	1.080	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

Table A 47 Weight height index kg (cm—100) for men and women in gr up 3

Age years	Men				Women			
	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg (cm—100	N	$\bar{x}$ kg cm—100	s kg 10	s kg cm —1
15—19	0	—	—		0			
20—24	0	—	—		2	0 8[illegible]		
25—29	0	—	—	—	0	—		
30—34	1	0 830	—		1	1 080		
35—39	1	1 330	—	—	1	0 910		
40—44	5	0 982	0 088	0 039[illegible]	5	0 926	0 06	0 [illegible]
45—49	10	1 036	0 117	0 03[illegible]0	8	1 01[illegible]	0 13	[illegible]
50—54	9	0 970	0 111	0 0370	6	0 [illegible]03	0 0[illegible]	
55—59	8	0 985	0 074	0 026	4	1 055	0 1[illegible]	
60—64	5	1 042	0 081	0 036	3	0 900	[illegible]	
65—69	2	0 910	0 028	0 0200	1	0 8[illegible]0		
70—74	2	0 930	0 098	0 0700	0	—		

N = number of subjects $\bar{x}$ = *mean* value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

Table A 48 Weight height index kg (cm—100) for men and women in group 4

Age years	Men				Women			
	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg (cm—100)	N	$\bar{x}$ kg (cm—100)	s kg cm—100	$s_{\bar{x}}$ kg (cm 100
15—19	0	—	—	—	0	—	—	—
20—24	0	—	—	—	3	1 136	0 046	0 0266
25—29	2	1 140	0 098	0 0700	0	—	—	—
30—34	1	1 200	—	—	0	—	—	—
35—39	15	1 146	0 065	0 0169	6	1 256	0 134	0 0549
40—44	38	1 161	0 064	0 0104	19	1 177	0 076	0 0176
45—49	32	1 161	0 073	0 0129	27	1 211	0 085	0 0163
50—54	25	1 169	0 081	0 0163	18	1 240	0 111	0 0262
55—59	15	1 183	0 094	0 0243	11	1 252	0 158	0 0477
60—64	6	1 201	0 064	0 0263	8	1 246	0 116	0 0411
65—69	3	1 146	0 032	0 0185	4	1 237	0 043	0 021[illegible]
70—74	0	—	—	—	0	—	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

Table A 49 Weight height index kg (cm—100) for men and women in group 5

Age years	Men				Women			
	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg/(cm—100)	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg/(cm—100)
15—19	0	—	—	—	0	—	—	—
20—24	0	—	—	—	0	—	—	—
25—29	0	—	—	—	0	—	—	—
30—34	1	0.990	—	—	0	—	—	—
35—39	0	—	—	—	0	—	—	
40—44	0	—	—	—	2	1.005	0.131	0.0950
45—49	3	0.910	0.009	0.0057	0	—	—	—
50—54	4	0.847	0.117	0.0586	2	1.105	0.091	0.0650
55—59	11	0.978	0.034	0.0255	0	—	—	—
60—64	2	0.960	0.141	0.1000	1	0.970	—	—
65—69	2	0.920	0.070	0.0500	0	—	—	—
70—74	1	0.870	—	—	0	—	—	—

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

Table A 50 Weight height index kg/(cm—100) for men and women in group 6

Age years	Men				Women			
	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg/(cm—100)	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg/(cm—100)
15—19	0				0		—	—
20—24	0				0			
25—29	0				0			
30—34	0				1	0.860		
35—39	0		—		1	0.970		
40—44	4	0.922	0.068	0.0342	4	1.076	0.099	0.0405
45—49	4	0.990	0.070	0.0353	4	0.990	0.099	0.0196
50—54	[illegible]	0.948	0.148	0.0660	3	0.913	0.092	0.0536
55—59	4	[illegible]	0.072	0.0361	4	1.030	0.069	0.0318
60—64	[illegible]	0.960	0.061	0.0275	3	1.020	0.086	0.0500
65—69	1	0.8[illegible]0			2	0.910	0.042	0.0300
70—74	0				0			

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

TABLE A 51 Weight height index kg/(cm—100) for men and women in group [illegible]

Age years	Men				Women			
	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg/(cm—100)	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg/(cm—100)
15—19	0	—	—	—	0			
20—24	0	—	—	—	[illegible]			
25—29	3	0.986	0.045	0.0[illegible]60				[illegible]344
30—34	4	0.887	0.135	0.0[illegible]6	4			[illegible]
35—39	9	0.938	0.143	0.04[illegible]8	1			0.03[illegible]3
40—44	19	0.931	0.08[illegible]	0.018[illegible]		[illegible].93		
45—49	17	0.915	0.097	0.0[illegible]3		0.9[illegible]	[illegible]	[illegible]
50—54	9	0.99[illegible]	0.151	0.0503		0.[illegible]	[illegible]18	0.03
55—59	10	0.965	0.081	0.0[illegible]5		[illegible]	[illegible]	[illegible]
60—64	4	0.935	0.0[illegible]1	0.0459	1	[illegible]0		
65—69	1	0.980	—		0			
70—74	0	—	—	—				

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

TABLE A 52 Weight height index kg/(cm—100) for men and women in group 9

Age years	Men				Women			
	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg/(cm—100)	N	$\bar{x}$ kg/(cm—100)	s kg/(cm—100)	$s_{\bar{x}}$ kg/(cm—100)
15—19	0	—	—		0			—
20—24	2	0.745	0.106	0.0[illegible]50	1	0.930		—
25—29	10	0.93[illegible]	0.108	0.034[illegible]	3	0.88[illegible]	0.181	0.1049
30—34	[illegible]	0.931	0.080	0.0378	8	0.855	0.061	0.0[illegible]1[illegible]
35—39	21	0.947	0.114	0.0[illegible]50	[illegible]	0.936	0.116	0.0[illegible]48
40—44	32	0.9[illegible]4	0.093	0.0164	[illegible]	0.9[illegible]3	0.108	0.0[illegible]17
45—49	24	0.931	0.105	0.0[illegible]16	18	0.914	0.128	0.0301
50—54	18	0.936	0.10[illegible]	0.0[illegible]40	17	0.95[illegible]	0.088	0.0[illegible]13
55—59	9	0.968	0.08[illegible]	0.0[illegible]4	8	0.965	0.093	0.0329
60—64	6	0.913	0.083	0.0341	3	0.890	0.112	0.0650
65—69	1	1.040	—		0		—	
70—74	0	—	—		0		—	

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

TABLE A 53 Serum cholesterol and ser m tr gl cer de con entra on an l log serum tr glycer le concentrat on for l eal hy tr en an l women (gro p 10 w h l fferent educat onal l ackgr un l

Ed ca t n	Age years	Men								
		N	Choles erol mg 100 m l			Tr gl cer des mn ole l			Tr glycer les log mmole l		
			$\bar{x}$	s	s	$\bar{x}$	s	s_x	—	s	s
Elem en tary sch o l	15—19	3	15? 7	5 8	3 3	0 80	0 06	0 03	—0 09b	0 030	0 018
	?0—?4	13	206 ?	27 8	7 7	1 0	0 40	0 11	—0 012	0 188	0 052
	?5—?9	18	242 1	43 8	10 3	1 29	0 51	0 12	0 085	0 139	0 033
	30—34	39	25? ?	57 4	9 ?	1 41	0 56	0 09	0 119	0 161	0 0 [illegible]
	35—39	94	?73 6	6? 4	[illegible] 1	1 47	0 64	0 07	0 1?8	0 180	0 019
	40—44	167	277 [illegible]	47 9	3 7	1 59	0 68	0 0	0 1[illegible]5	0 176	0 014
	4—49	162	?85 4	52 3	4 1	1 6[illegible]	0 78	0 0[illegible]	0 181	0 1 1	0 013
	50—54	14?	276 0	58 ?	4 9	1 49	0 [illegible]5	0 05	0 13	0 180	0 015
	55—59	90	284 7	49 4	5 ?	1 4	0 5?	0 0	0 134	0 145	0 015
	60 64	40	282 1	52 7	8 3	1 3?	0 37	0 0[illegible]	0 103	0 127	0 0?0
	[illegible] [illegible]9	1	?78 3	54	14 1	1 40	0 55	0 14	0 11[illegible]	0 167	0 043
	?0 4	3	2[illegible]3 0	13 1	7 6	0 74	0 08	0 0	—0 131	0 0 0	0 0?9
	1 4	8[illegible]	275 7	55 0	? 0	1 51	0 6[illegible]	0 0?	0 14?	0 172	0 00[illegible]
H gl s h o l	15 19	0	—	—	—		—	—	—	—	—
	20 ?4	1	2 5 0	—	—	0 74	—	—	0 131	—	—
	25 29	13	260 9	73 7	20 4	1 5[illegible]	0 77	0 21	0 143	0 ?16	0 0[illegible]0
	30 34	?5	247 6	48 1	9 [illegible]	1 33	0 5	0 11	0 0?8	0 180	0 036
	3 39	56	?64 ?	50 4	[illegible] 7	1 37	0 [illegible]2	0 03	0 102	0 171	0 0?3
	40—44	9	276 4	57 6	[illegible] 5	1 58	0 83	0 09	0 15	0 187	0 021
	4 49	78	287 1	48 4	5 5	1 44	0 6	0 0[illegible]	0 1?[illegible]	0 173	0 0?0
	50 54	47	268 1	49 4	7 ?	1 41	0 7	0 11	0 09	0 214	0 031
	55 59	26	?84 1	53 8	10 [illegible]	1 48	0 3	0 07	0 1 7	0 113	0 0 ?
	[illegible]0 [illegible]4	9	?61 4	4? 4	14 1	1 3	0 39	0 13	0 115	0 126	0 04?
	[illegible]5 [illegible]9	5	253 0	14 0	[illegible] ?	0 81	0 24	0 11	0 107	0 135	0 0[illegible]1
	0 74	1	?84 0	—		1 19		—	0 07		
	1 4	340	27? 8	5? 8	? 9	1 44	0 [illegible]	0 04	0 1?0	0 18?	0 010
A a le d g	15 19	0	—			—					
	0 ?4	1	?64 0			? 84			0 453	—	—
	?9	?	18? 0	[illegible]0 8	43 0	1 ?1	0 01	0 01	0 03?	0 005	0 001
	30 34	7	2?1 4	?8 1	10 [illegible]	1 1	0 39	0 15	0 044	0 173	0 0[illegible]
	35 39	??	259 7	37 4	7 0	1 47	0 8	0 1[illegible]	0 114	0 20[illegible]	0 038
	4 44	43	? 3 0	4? 1	[illegible] 4	1 51	0 6	0 12	0 141	0 171	0 0
	4 49	77	?83	5[illegible] 9	11 0	1 52	0 94	0 18	0 131	0 19[illegible]	0 038
	[illegible] 4	13	? 9[illegible]	[illegible] 1	18 3	1 59	0 80	0 ?	0 1 9	0 194	0 0 4
		10	? ? 0	43 ?	13 7	1 0?	0 40	0 13	-0 03?	0 ?17	0 0[illegible]9
	4	[illegible]	?7 ?	4 5	19 0	1 ?5	0 33	0 13	0 03?	0 116	0 04?
	9	1	310 0			1 41			0 149		
	4	1	5 0			0 89			0 0 1		
	4	140	? ? 3	48 9	4 1	1 4	0 8	0 07	0 115	0 184	0 01[illegible]

N num er f t x m an al e s s an la l d a on s s an lar l err r of t e m an

Women

N	Cholesterol mg/100 ml			Triglycerides mmole l					
	$\bar{x}$	s	s	$\bar{x}$	s				
8	223 5	25 1	8 9	0 91	0 1				
35	221 3	45 3	7 7	0 93	0				
37	228 7	53 0	8 7	1 00					
30	255 0	44 8	8 2	1 03					
103	264 4	45 8	4 5	1 16					
240	267 7	53 6	3 5	1 07					
180	290 0	54 7	4 1	1 26					
138	303 5	56 [illegible]	4 8	1 28	4				[illegible]
58	311 6	59 8	7 9	1 27				4	116
15	3?5 5	53 0	13	1 ?2				5	
3	274 7	63 8	36 8	1 08	0 3			7	[illegible]
1	49? 0	—	—	1 38					
848	277 7	58 5	2 0	1 16	0 4	[illegible]	4	16	0 0
0	—	—	—	—					
4	193 3	74 6	37 3	0 71	0 19	10	[illegible]	0 114	0 05
0	—	—	—	—					—
2	236 0	18 4	13 0	2 05	0 27	0 19	0 5 [illegible]	0 057	0 343
5	312 6	131 5	58 8	0 94	0 17	0 0	0 034	0 085	0 ?38
26	252 3	56 9	11 ?	1 03	0 4?	0 08	0 015	0 149	0 0 9
26	281 8	54 2	10 6	1 07	0 40	0 06	0 001	0 164	0 03?
11	308 1	50 1	15 1	1 30	0 46	0 14	0 093	0 141	0 043
9	279 3	29 2	9 7	1 ?0	0 29	0 10	0 068	0 09	0 032
3	357 7	49 9	28 8	1 21	0 37	0 ?2	0 065	0 148	0 086
1	290 0	—	—	1 00	—		0 000	—	
0	—	—	—					—	
87	275 4	64 9	7 0	1 10	0 4?	0 04	0 015	0 154	0 01
0	—	—	—		—		—		
0	—	—	—			—			
0	—	—	—	—	—			—	—
0	—	—	—		—		—	—	
4	264 8	40 0	20 0	0 94	0 27	0 14	−0 047	0 142	0 071
3	269 3	7 1	4 1	0 79	0 20	0 11	0 114	0 107	0 06?
2	250 5	12 0	8 5	0 64	0 04	0 03	−0 195	0 028	0 070
3	262 0	16 1	9 3	1 09	0 39	0 23	0 013	0 1 6	0 10?
2	38? 5	111 0	78 5	1 64	0 35	0 20	0 208	0 09?	0 066
0	—	—	—		—				—
0	—	—	—	—				—	—
0	—	—	—		—		—	—	—
14	279 9	57 4	15 3	1 00	0 39	0 10	−0 03?	0 164	0 044

l prese re f r healthy men and wor en (group 10

kir nd

					Systolic blood pressure mm Hg		Diastolic blood pressure mm Hg	
					$\bar{x}$	s_x	$\bar{x}$	s
					1?3	8 8	86 7	?8
					11 8	3 3	80 0	6 5
					9 0	2 1	79 4	4 6
					10 6	1 7	81 3	7 4
					11 5	1 2	84 8	8 4
					[illegible]	1 1	[illegible]	[illegible]
					13 [illegible]	1 1	8[illegible] 3	8 0
					11 7	1 2	87 1	7[illegible]
					13 5	1 4	87 7	[illegible]
					[illegible]	2 3	87 4	8 0
					12 6	3 3	84 3	8 4
					8 7	6 0	90 0	5 8
					14 1	0 5	8[illegible] 6	8 1
					—	—	—	—
					—	—	90 0	—
					9 2	2 5	[illegible]	7 0
					13 5	2 7	[illegible]	[illegible]
					13 3	1 8	[illegible]	8 4
	4 45				12 7	1 4	84 6	8 0
	4 4				[illegible]	1 5	[illegible]	8 3
	1	4			[illegible]	2 1	[illegible]	8 8
	[illegible]				[illegible]	2 8	87 1	7 6
	[illegible] 4				13 2	4 4	[illegible]	8 7
	[illegible]				14 0	[illegible]	85 0	7 1
	4	1	1			—	100 0	—
	[illegible] 4	[illegible]	[illegible]		13 5	[illegible]	[illegible]	9 3
[illegible]	[illegible] 19	6				—	—	—
[illegible]	[illegible]	1	[illegible]			—	80 0	—
	[illegible]	[illegible]	[illegible]		7 1	5 0	85 0	—
	[illegible] 31	7	[illegible]		13 5	5 1	81 4	8 5
	[illegible]	[illegible]	[illegible]		[illegible]	1 4	[illegible]	8 5
	[illegible] 44	43	[illegible]		9 9	1 5	[illegible]	[illegible]
	[illegible] 49	[illegible]	[illegible]		12 6	2 4	[illegible]	6 8
	[illegible]	13	[illegible]		11 5	3 7	[illegible]	8 3
	[illegible] 51	10	[illegible]		[illegible]	3 7	83 8	5
	[illegible]	1	[illegible]		12 4	[illegible] 1	84 2	3 8
	[illegible]	1	[illegible]			—	90 0	—
	[illegible]	1	[illegible]				90 0	—
	[illegible]	[illegible]	[illegible]		12 4	1 0	[illegible]	[illegible]

[illegible] standard [illegible] standard error of the [illegible]

men

	Systoli blood pressure mm Hg			Diast[illegible] mm Hg		
	$\bar{x}$	s	s_x	$\bar{x}$		
	120 0	5 3	1 9	76 3		
	120 3	6 1	1 0	75 6		
	122 0	12 2	2 0	77 2	8	
	121 2	10 0	1 8	7[illegible] 2		
	127 5	12 8	1 3	81 1		[illegible]
	130 7	1[illegible] 0	0 9	82 6		[illegible]
	133 1	13 5	1 0	83 2		
	139 9	14 7	1 3	85 6	9	[illegible]
	144 7	15 2	2 0	83 2		
	151 0	18 1	4 7	90 7		
	145 0	13 2	7 6	88 3		4 4
	170 0	—	—	95 0		
	137 5	15 2	0 5	87 9	8	[illegible]
	—	—	—	—		
	115 0	10 0	5 0	72 5	15 0	[illegible]
	—	—	—	—		
	122 5	3 5	2 5	75 0	1	5 0
	138 0	10 4	4 6	87 0	6 7	3 0
	125 4	9 0	1 8	78 5	6 4	1 3
	130 0	13 9	2 7	79 6	7 6	1 5
	128 2	12 1	3 6	82 7	6 8	2 1
	146 1	12 4	4 1	87 8	6 7	2 2
	151 7	20 2	11 7	91 7	2 9	1 7
	160 0	—	—	90 0		—
	—	—	—	—	—	
	130 7	14 3	1 5	81 0	8 3	0 9
	—	—		—	—	—
	—	—	—	—	—	—
	—	—	—	—	—	
	—	—	—	—	—	—
	127 5	15 0	7 5	82 5	10 4	5 2
	126 7	7 6	4 4	85 0	5 0	2 9
	130 0	7 1	5 0	75 0	[illegible] 1	5 0
	128 3	10 4	6 0	81 7	12 6	7 3
	150 0	7 1	5 0	92 5	10 6	7 5
0	—	—	—	—		—
0	—	—	—	—	—	—
0	—	—	—	—	—	—
14	131 5	12 3	3 3	83 2	9 5	2 5

Table A 35 Weight height index kg (cm — 100) for healthy men and women (group 10) with different educational background

Education	Age years	Men			
		N	$\bar{x}$ kg (cm — 100)	s kg (cm — 100)	$s_{\bar{x}}$ kg (cm — 100)
Elementary school	15—19	3	0.810	0.045	0.0264
	20—24	13	0.870	0.094	0.0263
	25—29	18	0.890	0.101	0.0239
	30—34	39	0.914	0.082	0.0132
	35—39	94	0.938	0.097	0.0100
	40—44	167	0.916	0.120	0.0093
	45—49	162	0.930	0.090	0.0071
	50—54	142	0.943	0.115	0.0097
	55—59	90	0.951	0.128	0.0134
	60—64	40	0.958	0.123	0.0194
	65—69	15	0.934	0.121	0.0313
	70—74	3	0.926	0.032	0.0185
	15—74	785	0.943	0.110	0.0039
High school	15—19	0	—	—	—
	20—24	1	0.850	—	—
	25—29	13	0.880	0.072	0.0200
	30—34	25	0.909	0.092	0.0185
	35—39	56	0.895	0.130	0.0174
	40—44	79	0.928	0.084	0.0094
	45—49	78	0.933	0.081	0.0092
	50—54	47	0.951	0.098	0.0143
	55—59	26	0.915	0.069	0.0136
	60—64	9	0.899	0.081	0.0271
	65—69	5	0.852	0.093	0.0412
	70—74	1	0.940	—	—
	15—74	349	0.923	0.095	0.0051
Vocational centre	15—19	0	—	—	—
	20—24	1	0.999	—	—
	25—29	2	0.885	0.071	0.0149
	30—34	7	0.854	0.119	0.0452
	35—39	29	0.914	0.083	0.0154
	40—44	43	0.946	0.079	0.0121
	45—49	27	0.933	0.063	0.0122
	50—54	13	0.893	0.069	0.0191
	55—59	10	0.933	0.118	0.0373
	60—64	6	0.970	0.031	0.0129
	65—69	1	1.099	—	—
	70—74	1	0.840	—	—
	15—74	140	0.928	0.073	0.0060

N = number of cases; $\bar{x}$ = mean value; s = standard deviation; $s_{\bar{x}}$ = standard error of the mean

Women			
N	x kg (cm — 100)	s kg (cm — 100	kg
8	0 860	0 153	
35	0 848	0 084	0
37	0 877	0 114	0
30	0 924	0 091	0
103	0 926	0 097	
240	0 940	0 093	
180	0 943	0 111	
138	0 960	0 101	0
58	0 963	0 098	
15	1 0?3	0 108	
3	0 96	0 208	
1	1 030	—	
848	0 938	0 104	0 3
0	—	—	
4	0 837	0 017	0 00 5
0		—	
2	0 975	0 007	0 (50
5	0 896	0 088	0 019
26	0 901	0 094	018
26	0 9?3	0 093	0183
11	0 928	0 068	0 02
9	0 965	0 074	0 1
3	0 873	0 015	0 0 83
1	0 9 0	—	
0	—	—	
87	0 916	0 08	( )
0	—		
0			
0	—		
0	—		
4	0 905	0 081	0 010
5	0 863	0 10	0 (
2	0 935	0 162	11)
3	1 013	0 015	0 0 8
2	0 975	0 007	0 0( )
0	—		
0			
0	—		
14	0 942	0 031	((2 2

TABLE X.55 Weight height index kg/(cm − 100) for healthy men and women (group 10) with different educational background

Education	Age years	Men			
		N	$\bar{x}$ kg/(cm − 100)	s kg/(cm − 100)	$s_{\bar{x}}$ kg/(cm − 100)
Elementary school	15–19	3	0.810	0.045	0.0264
	20–24	13	0.8[illegible]0	0.094	0.0263
	25–29	18	0.830	0.101	0.0239
	30–34	39	0.914	0.082	0.0132
	35–39	94	0.939	0.097	0.0100
	40–44	167	0.916	0.120	0.0093
	45–49	162	0.9[illegible]0	0.093	0.0071
	50–54	142	0.943	0.115	0.0097
	55–59	93	0.951	0.128	0.0134
	60–64	49	0.958	0.123	0.0194
	65–69	15	0.934	0.121	0.0313
	70–74	3	0.976	0.032	0.0185
	15–74	785	0.943	0.110	0.0039
High school	15–19	0	—	—	—
	20–24	1	0.850	—	—
	25–29	13	0.880	0.072	0.0200
	30–34	25	0.909	0.092	0.0185
	35–39	56	0.895	0.130	0.0174
	40–44	79	0.928	0.084	0.0094
	45–49	79	0.933	0.031	0.0093
	50–54	47	0.951	0.098	0.0143
	55–59	26	0.945	0.069	0.0136
	60–64	9	0.890	0.031	0.0271
	65–69	5	0.8[illegible]2	0.093	0.0442
	70–74	1	0.940	—	—
	15–74	349	0.923	0.095	0.0051
Academic degree	15–19	0	—	–	—
	20–24	1	0.999		—
	25–29	2	0.835	0.021	0.0149
	30–34	7	0.854	0.119	0.0[illegible]2
	35–39	29	0.914	0.083	0.0154
	40–44	43	0.915	0.0[illegible]9	0.0121
	45–49	27	0.939	0.0[illegible]3	0.0172
	50–54	15	0.893	0.0[illegible]9	0.0191
	55–59	10	0.933	0.118	0.0373
	60–64	6	0.9[illegible]0	0.031	0.0129
	65–69	1	1.0[illegible]		—
	70–74	1	0.810	—	—
	15–74	140	0.9[illegible]8	0.0[illegible]1	0.0070

N = number of subjects, $\bar{x}$ = mean value, s = standard deviation, $s_{\bar{x}}$ = standard error of the mean

Women

\	Cholesterol mg/100 ml			Triglycerides mmole/l					
	$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	s			
8	223 5	25 1	8 9	0 91	0 17	0			
39	218 4	48 5	7 8	0 96	0 29	0 0			
37	228 7	53 0	8 7	1 00	0 34	0 0			
30	253 1	45 0	8 2	1 06	0 51	0 09		+	(3
107	266 9	52 3	5 1	1 15	0 61	0 16	(		0)1
248	265 2	52 9	3 4	1 06	0 41	0 03	—0 0)2	(	0)10
193	286 9	54 2	3 9	1 24	0 50	0 04	0 0F1	(6	0 012
143	303 6	56 2	4 8	1 27	0 44	0 14	0 031	(14	0 01?
59	307 5	61 6	8 0	1 31	0 61	0 08	0 097	0 14	0 019
15	321 8	50 1	12 9	1 21	0 39	0 10	0 0F1	0 133	0 034
4	278 5	52 7	26 3	1 06	0 27	0 14	0 014	0 120	0 060
1	492	—	—	1 38	—	—	0 13ɟ		—
831	276 0	58 9	2 0	1 16	0 48	0 02	0 032	0 160	0 005
0	—	—	—			—	—	—	—
0	—	—	—	—		—			—
0	—	—	—	—		—		—	—
2	264 5	6 4	4 5	1 60	1 11	0 78	0 142	0 330	0 234
5	259 8	48 4	21 7	1 01	0 18	0 08	0 001	0 075	0 034
21	278 3	62 1	13 5	1 08	0 46	0 10	—0 024	0 281	0 061
15	310 6	53 8	13 9	1 07	0 41	0 11	—0 001	0 166	0 043
12	295 8	46 2	13 3	1 34	0 48	0 14	0 100	0 158	0 046
10	321 2	48 0	15 2	1 05	0 16	0 05	0 016	0 070	0 022
3	376 0	4ɔ 9	‹7 1	1 29	0 44	0 25	0 091	0 168	0 097
0	—	—	—	—		—	—	—	—
0	—	—	—			—	—	—	—
68	297 5	57 2	6 9	1 14	0 43	0 05	0 020	0 199	0 024

Table 5. Systolic and diastolic blood pressure of healthy persons by age group (30) in subordinate and supervisory positions

Position	Age years	Men N	Systolic blood pressure mm Hg $\bar{x}$	s	$s_{\bar{x}}$	Diastolic blood pressure mm Hg $\bar{x}$	s	$s_{\bar{x}}$	Women N	Systolic blood pressure mm Hg $\bar{x}$	s	$s_{\bar{x}}$	Diastolic blood pressure mm Hg $\bar{x}$	s	$s_{\bar{x}}$
Subordinate	15–19	3	133.3	15.3	8.8	8[illegible].7	[illegible]	3.4	8	1[illegible]0.0	5.3	1.[illegible]	7[illegible].3	4.4	1.6
	20–24	13	1[illegible]8.4	10	2.[illegible]	80.0	[illegible]	1.8	39	111.7	[illegible].6	1.1	7[illegible].3	[illegible].7	1.1
	25–29	[illegible]	131.[illegible]	[illegible]3	1.8	[illegible]	[illegible].1	1.2	3	1[illegible]2.0	12.2	2.0	77.2	8.8	1.4
	30–34	47	1[illegible]8.[illegible]	13.1	1.[illegible]	81.3	7.4	1.1	30	1[illegible]0.0	8.[illegible]	1.6	7[illegible].2	5.7	1.0
	35–39	11[illegible]	130.5	11.[illegible]	1.1	83.2	8.0	0.7	107	127.[illegible]	12.6	1.2	81.1	7.8	0.8
	40–44	168	133.1	13.[illegible]	1.1	83.3	7.8	0.6	248	130.0	13.7	0.9	82.1	7.9	0.5
	45–49	1[illegible]8	135.[illegible]	12.5	1.0	85.7	7.[illegible]	0.6	193	133.2	13.0	0.[illegible]	82.9	7.7	0.6
	50–54	129	13[illegible].2	14.6	1.3	8[illegible].3	7.5	0.7	140	140.0	14.8	1.3	85.5	8.5	0.7
	55–59	[illegible]3	141.0	13.2	1.[illegible]	88.1	7.4	0.[illegible]	9	145.2	11.1	1.8	81.2	7.1	0.9
	60–64	5[illegible]	14[illegible].5	13.9	2.3	8[illegible].9	8.0	1.3	1[illegible]	1[illegible]0.0	17.4	4.[illegible]	90.3	[illegible].9	1.8
	65–69	15	141.0	12.8	3.3	83.3	8.4	2.2	4	148.8	13.1	[illegible].6	88.8	[illegible].3	3.1
	70–74	[illegible]	1[illegible]3.0	7.[illegible]	3.4	9[illegible].0	[illegible].7	[illegible]	1	170.0	—	—	95.0		
	15–74	[illegible]	13[illegible].0	13.8	0.[illegible]	81.6	7.9	0.3	881	132.1	1[illegible].0	0.[illegible]	8[illegible].6	8.4	0.3
Supervisory	15–19	0				—			0		—		—		
	20–24	2	140.0	14.[illegible]	10.0	85.0	7.[illegible]	[illegible].0	0		—	—	—	—	—
	25–29	6	1[illegible]8.3	[illegible].8	2.8	79.2	9.7	4.0	0				—		
	30–34	24	131.[illegible]	[illegible].4	1.9	8[illegible].1	[illegible].4	1.[illegible]	2	140.0			90.0		
	35–39	[illegible]3	13[illegible].0	13.8	1.7	84.5	9.2	1.2	[illegible]	138.0	11.4	[illegible].4	88.0	7.6	3.4
	40–44	1[illegible]1	13[illegible].1	13.0	1.2	8[illegible].6	8.0	0.7	21	133.8	12.4	2.7	84.0	10.1	2.2
	45–49	109	13[illegible].3	14.[illegible]	1.4	8[illegible].8	8.6	0.8	15	1[illegible].3	17.7	4.6	[illegible]9.7	10.6	2.7
	50–54	[illegible]3	13[illegible].0	14.6	1.7	8[illegible].8	8.7	1.0	12	130.0	11.3	3.3	83.3	8.9	2.[illegible]
	55–59	[illegible]3	13[illegible].2	14.3	2.0	8[illegible].0	8.0	1.1	10	144.0	18.0	[illegible]	88.[illegible]	10.0	3.2
	60–64	18	13[illegible].1	1[illegible].0	3.8	8[illegible].1	7.4	1.7	3	1[illegible].7	23.1	13.3	93.3	2.[illegible]	1.[illegible]
	65–69	[illegible]	133.3	13.3	5.4	89.3	[illegible].2	2.1	0	—					
	70–74	0						—	0						—
	15–74	4[illegible]5	13[illegible].1	13.9	0.6	8[illegible].0	8.4	0.4	[illegible]8	131.5	1[illegible].1	2.0	84.5	9.[illegible]	1.2

N = number of subjects; $\bar{x}$ = mean value; s = standard deviation; $s_{\bar{x}}$ = standard error of the mean

TABLE A 58 Weight height index for healthy m[illegible]n and wom[illegible]n [illegible] supervisory pos[illegible]tions

Po[illegible]on	Age years	Men N	$\bar{x}$ kg/(cm — 100)	s kg/(cm 1(0)	s kg/(cm 10))	Wom[illegible] N			
Sub-ordinate	15—19	3	0 810	0 04[illegible]	0 0 [illegible]4	8	0 8 [illegible]	[illegible]	
	20—24	13	0 8 0	0 08[illegible]	0 0[illegible]40	39	0 84	[illegible] 0	0
	[illegible]5—29	[illegible]7	0 875	0 089	0 01	37	0 [illegible]	114	0
	30—34	47	0 893	0 088	0 0128	30	0 9	0 9	0 0
	3[illegible]—39	116	0 917	0 115	0 0107	10	0 [illegible]	[illegible] 0	0 4
	40—44	168	0 93[illegible]	0 09[illegible]	0 00 4	248	0 9 [illegible]	0[illegible]	0 1
	45—49	1[illegible]8	0 946	0 091	0 007	193	0 94[illegible]	111	
	50—54	1[illegible]9	0 936	0 116	0 010[illegible]	140	0 9[illegible]	0 100	[illegible]
	55 59	73	0 9[illegible]6	0 077	0 0090	59	0 9[illegible]	0 0[illegible]9	0 [illegible]116
	60—64	37	0 948	0 113	0 018[illegible]	15	1 014	0 113	0 0 [illegible]4
	6[illegible]—69	1[illegible]	0 9 9	0 110	0 0 85	4	0 [illegible]67	0 169	0 084[illegible]
	70—74	5	0 912	0 046	0 0 08	1	1 030	—	—
	15—74	*791*	*0 931*	*0 101*	*0 0036*	*881*	*0 934*	*0 104*	*0 003[illegible]*
Supervisory	15—19	0	—	—	—	0	—	—	
	20—24	[illegible]	0 9[illegible]5	0 162	0 1150	0	—	—	—
	25—29	6	0 903	0 070	0 0289	0	—	—	
	30—34	24	0 931	0 092	0 0187	2	0 945	0 120	0 0349
	35—39	63	0 9[illegible]6	0 094	0 0119	5	0 93[illegible]	0 067	0 030
	40—44	1[illegible]1	0 9[illegible]4	0 118	0 0107	21	0 941	0 065	0 014[illegible]
	45—49	109	0 9[illegible]	0 079	0 0075	15	0 948	0 07[illegible]	0 019[illegible]
	50—54	73	0 951	0 097	0 0114	1[illegible]	0 98[illegible]	0 0[illegible]	0 0180
	55—59	53	0 937	0 155	0 0[illegible]14	10	1 004	0 109	0 0344
	60—64	18	0 948	0 114	0 0269	3	0 916	0 089	0 0[illegible]17
	65—69	6	0 911	0 154	0 0628	0	—	—	—
	70—74	0	—	—	—	0	—	—	—
	15—74	475	0 946	0 107	0 0049	68	0 9[illegible]7	0 078	0 0094

N number of subjects x = mean [illegible]alue s standard de[illegible]iation $s_{\bar{x}}$ standard error of the mean

TABLE A 39 Serum-cholesterol and serum triglyceride concentration and log serum triglyceride concentration for healthy men and women (group 10) with different degrees of physical activity at work

Physical activity	Age years	Men N	Cholesterol mg 100 ml $\bar{x}$	s	$s_{\bar{x}}$	Triglycerides mmole l $\bar{x}$	s	$s_{\bar{x}}$	Triglycerides log mmole l $\bar{x}$	s	$s_{\bar{x}}$
None	15–19	1	146.0	—	—	0.83	—	—	−0.031	—	—
	20–24	11	215.5	35.8	10.8	1.16	0.70	0.21	−0.002	0.247	0.075
	25–29	30	245.9	62.3	11.4	1.47	0.63	0.12	0.116	0.170	0.031
	30–34	50	230.5	55.9	7.9	1.39	0.57	0.08	0.110	0.165	0.023
	35–39	124	262.6	51.9	4.7	1.46	0.70	0.06	0.123	0.183	0.016
	40–44	184	277.1	50.6	3.7	1.62	0.78	0.06	0.167	0.186	0.014
	45–49	164	281.0	52.6	4.1	1.60	0.83	0.06	0.161	0.189	0.015
	50–54	103	277.9	54.7	5.4	1.51	0.79	0.08	0.131	0.200	0.020
	55–59	64	285.4	47.4	5.9	1.42	0.57	0.07	0.117	0.173	0.022
	60–64	27	274.7	45.9	8.8	1.37	0.36	0.07	0.121	0.115	0.022
	65–69	11	2[illegible]5.5	43.9	13.2	1.16	0.51	0.15	0.077	0.183	0.055
	70–74	4	261.8	26.3	13.2	0.89	0.23	0.11	−0.0[illegible]	0.103	0.051
	15–74	773	2[illegible]2	53.3	1.9	1.51	0.73	0.03	0.13[illegible]	0.186	0.007
Moderate	15–19	2	156.0	—	—	0.79	0.07	0.05	−0.104	0.038	0.028
	20–24	4	207.5	20.4	10.2	1.14	0.23	0.12	0.047	0.093	0.047
	25–29	3	215.0	12.1	7.0	1.11	0.46	0.26	0.074	0.165	0.09[illegible]
	30–34	21	247.[illegible]	42.8	9.3	1.29	0.49	0.11	0.0[illegible]9	0.1[illegible]8	0.039
	35–39	50	291.8	63.0	8.9	1.40	0.6[illegible]	0.09	0.103	0.129	0.0[illegible]
	40–44	100	273.9	47.6	4.8	1.50	0.63	0.06	0.144	0.164	0.016
	45–49	99	293.5	49.5	5.0	1.35	0.58	0.06	0.1[illegible]	0.153	0.015
	50–54	92	2[illegible]0.7	57.5	6.0	1.4[illegible]	0.56	0.06	0.1[illegible]	0.1[illegible]	0.018
	55–59	53	283.6	54.7	7.5	1.43	0.40	0.05	0.137	0.130	0.018
	60–64	28	281.5	54.8	10.4	1.27	0.36	0.07	0.085	0.131	0.02[illegible]
	65–69	9	2[illegible]8.3	53.7	17.9	1.40	0.60	0.20	0.103	0.189	0.0[illegible]3
	70–74	1	251.0	—	—	0.77	—	—	−0.114	—	
	15–74	4[illegible]	27[illegible].1	54.6	2.5	1.45	0.5[illegible]	0.03	0.129	0.1[illegible]2	0.008
Hard	15–19	0	—	—	—	—	—		—	—	—
	20–24	0	—	—	—	—	—	—	—	—	—
	25–29	0		—		—	—	—	—		—
	30–34	0				—	—	—	—	—	—
	35–39	5	2[illegible]2	43.1	19.3	1.33	0.65	0.29	0.03[illegible]	0.1[illegible]	0.031
	40–44	5	313.8	6[illegible].7	[illegible]8.0	1.55	0.67	0.30	0.157	0.189	0.0[illegible]5
	45–49	4	2[illegible]3.3	3[illegible].8	18.4	1.68	0.77	0.39	0.190	0.207	0.104
	50–54		[illegible]7	8.5	29.7	1.3[illegible]	0.67	0.25	0.054	0.[illegible]4	0.0[illegible]
	55–59	9	[illegible]0.1	34.3	11.4	1.37	0.4[illegible]	0.14	0.119	0.130	0.043
	60–64	0			—			—			
	65–69	1	[illegible]14.0			1.[illegible]	—		0.0[illegible]	—	—
	70–74	0					—	—			—
	15–74	31	[illegible]	54.1	9.7	1.43	0.[illegible]7	0.10	0.1[illegible]	0.172	0.031

N = number of [illegible] $\bar{x}$ = mean, s = standard deviation, $s_{\bar{x}}$ = standard error of the mean

Women

N	Cholesterol mg/100 ml			Triglycerides mmole/l					
	$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$			
8	223 5	25 1	8 9	0 91	0 1[illegible]	0 [illegible]	[illegible]	[illegible]	[illegible]
32	212 6	48 4	8 6	0 91	0 2	0 05	[illegible]	[illegible]	[illegible]
33	230 5	55 0	9 6	1 00	0 35	0 06	[illegible]	[illegible]	[illegible]
24	251 5	49 0	10 0	0 96	0 46	0 0[illegible]	[illegible]	0 179	0 03
95	266 7	52 4	5 4	1 11	0 40	0 04	[illegible]	138	0 014
705	267 2	54 0	3 8	1 06	0 42	0 05	[illegible]	[illegible]	0 011
149	284 9	51 4	4 2	1 23	0 43	0 04	[illegible]	0 162	0 013
113	305 7	55 3	5 2	1 26	0 41	0 04	[illegible]	0 135	0 013
45	313 5	58 9	8 8	1 30	0 65	0 10	0 083	0 145	0 022
11	330 8	57 5	17 3	1 30	0 40	0 12	0 095	0 125	0 038
1	290 0	—	—	1 00	—		0 000	—	—
1	492 0	—	—	1 38	—	—	0 139	—	—
717	276 0	59 2	2 2	1 14	0 45	0 02	0 076	0 156	0 006
0	—	—	—	—	—	—	—	—	—
6	231 5	24 0	9 8	1 14	0 19	0 08	0 050	0 075	0 031
4	214 3	33 0	16 5	1 03	0 26	0 13	0 001	0 102	0 051
8	260 [illegible]	22 0	7 8	1 49	0 63	0 22	0 134	0 204	0 072
17	266 2	50 3	12 2	1 36	1 24	0 30	0 053	0 235	0 057
62	262 6	53 0	6 7	1 06	0 39	0 05	—0 010	0 201	0 076
53	296 4	61 2	8 4	1 23	0 52	0 07	0 051	0 182	0 025
34	296 1	56 3	9 7	1 38	0 54	0 09	0 111	0 157	0 027
23	301 3	63 0	13 1	1 21	0 39	0 08	0 067	0 121	0 025
7	330 9	47 9	18 1	1 10	0 36	0 13	0 020	0 144	0 055
3	274 7	63 8	36 8	1 08	0 33	0 19	0 019	0 147	0 085
0	—	—	—	—	—	—	—	—	—
217	281 1	58 4	4 0	1 21	0 56	0 04	0 045	0 182	0 012
0	—	—	—	—	—	—	—	—	—
1	327 0	—	—	1 39	—	—	0 143	—	—
0	—	—	—	—		—	—	—	—
0	—	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—	—
2	277 5	74 2	52 5	1 40	0 16	0 11	0 143	0 050	0 036
6	313 3	61 5	25 1	1 37	0 72	0 23	0 088	0 223	0 091
5	288 4	55 5	24 8	0 99	0 26	0 11	—0 017	0 131	0 059
1	316 0	—	—	1 13	—	—	0 053	—	—
0	—	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—	—
15	301 3	53 7	13 9	1 23	0 49	0 13	0 0[illegible]1	0 163	0 042

TABLE X[illegible] Weight/height index (kg/cm — 100) for healthy men and women [illegible] with different degrees of physical activity at work

Physical activity	Age years	Men			
		N	$\bar{x}$ kg/cm — 100	s kg/cm — 100	s kg/cm — 100
None	15–19	[illegible]	[illegible]	—	—
	20–24	11	0.8[illegible]8	0.0[illegible]4	0.0[illegible]5
	25–29	39	0.820	0.0[illegible]	0.0158
	30–34	5[illegible]	0.903	0.097	0.0138
	3[illegible]–39	1[illegible]4	0.9[illegible]	0.1[illegible]	0.010[illegible]
	40–44	18[illegible]	0.936	0.0[illegible]0	0.00[illegible]9
	4[illegible]–4[illegible]	1[illegible]4	0.931	0.097	0.00[illegible]9
	50–54	103	0.9[illegible]	0.11[illegible]	0.0113
	55–59	[illegible]4	0.93[illegible]	0.137	0.0171
	60–64	[illegible]	0.915	0.103	0.0199
	6[illegible]–69	11	0.90[illegible]	0.140	0.0[illegible]4
	[illegible]0–[illegible]4	4	0.90[illegible]	0.050	0.0[illegible]53
	15–[illegible]4	73	0.930	0.10[illegible]	0.003[illegible]
Moderate	1[illegible]–19	[illegible]	0.835	0.07[illegible]	0.0149
	[illegible]0–[illegible]4	4	0.90[illegible]	0.144	0.0[illegible]1
	[illegible]5–[illegible]9	3	0.8[illegible]6	0.09[illegible]	0.0[illegible]9
	30–34	[illegible]1	0.901	0.071	0.015[illegible]
	3[illegible]–39	[illegible]	0.91[illegible]	0.09[illegible]	0.0129
	40–44	100	0.947	0.141	0.0141
	45–49	[illegible]	0.944	0.0[illegible]8	0.00[illegible]8
	50–[illegible]4	[illegible]	0.934	0.09[illegible]	0.0103
	[illegible]5–59	53	0.9[illegible]4	0.079	0.0109
	60–64	[illegible]8	0.9[illegible]	0.1[illegible]	0.0[illegible]3[illegible]
	[illegible]–[illegible]9	9	0.953	0.0[illegible]3	0.0329
	[illegible]0–[illegible]4	1	0.940	—	—
	15–[illegible]4	[illegible]	0.945	0.104	0.0018
H[illegible]	15–19	0			—
	[illegible]0–[illegible]4	0			
	[illegible]5–[illegible]9	0		—	
	30–34	0			
	[illegible]–39	5	0.93[illegible]	0.134	0.0[illegible]
	4[illegible]–44	5	1.0[illegible]	0.113	0.0[illegible]
	4[illegible]–49	4	1.03	0.1[illegible]	0.0[illegible]
	[illegible]–[illegible]4	[illegible]	1.001	0.145	0.0549
	[illegible]	[illegible]	0.913	0.1[illegible]7	0.01[illegible]
	60–[illegible]4	0			
	[illegible]5–[illegible]	1	0.8[illegible]0		
	[illegible]4	0			
	1[illegible]–[illegible]4	31	0.9[illegible]	0.135	0.0[illegible]

[illegible] standard error of the mean

Women			
N	$\bar{x}$ kg/(cm — 100)	s kg/(cm — 100)	s_x kg (cm — 100)
8	0 860	0 153	0 0543
37	0 839	0 087	0 0145
33	0 881	0 115	0 0200
24	0 936	0 087	0 0178
95	0 976	0 098	0 0100
205	0 930	0 095	0 0066
149	0 941	0 115	0 0094
113	0 944	0 087	0 0082
45	0 960	0 097	0 0138
11	0 983	0 060	0 0181
1	0 970	—	—
1	1 030	—	—
717	0 930	0 107	0 0038
0	—	—	—
6	0 865	0 036	0 0150
4	0 850	0 124	0 0621
8	0 900	0 094	0 0334
17	0 908	0 085	0 0208
62	0 952	0 086	0 0110
53	0 931	0 084	0 0115
34	0 991	0 114	0 0196
23	0 969	0 096	0 0202
7	1 071	0 172	0 0653
3	0 966	0 208	0 1201
0	—	—	—
217	0 948	0 101	0 0068
0	—	—	—
1	1 000	—	—
0			—
0	—	—	—
0	—	—	—
2	1 010	0 127	0 0899
6	01	0 151	0 0618
5	1 66	0 109	0 0488
1	0	—	—
0		—	
0		—	—
0		—	—
15	1 0 8	0 116	0 0301

Tar a XC? S r cl bery l a l s n r c cn rs n anl bgar nt gl c r l
mern a e f r l l s n n a l w n n g n 10 rew e n 0 rent leg es
f gl scal a w k

In al a	A years	Men									
		N	Ol d s l g l n l			Tr gl cer les l l			Tr gl cer les l og m ol e l		
			x	s	s	$\bar{x}$	s	s	x	s	s
N	1> 19	1	14		—	0 83			—0 f31	—	
	20 4		3 0	1	1 9	1 73	0 74	0 17	0 095	0 03	0 0 0
	5	11		0	1 9	1 1	0 8	0 73	0 159	0 ?	0 0
	3 33	3	13 8		17 (	1 49	0 44	0 09	0 17	0 1 8	0 0 7
	35 3	(	0 4		7	1	0 f3	0 08	0 15	0 1 1	0 0 ?
	4 44	3			1	1 7	0 93	0 11	0 194	0 71	0 0 5
	4 4		° 3	4	f ?	1 f9	0 7	0 19	0 191	0 1 9	0 0 0
	4	1		8	7 8	1 53	0 73	0 09	0 15°	0 193	0 0 5
	?	41	1	3	5	1 55	0	0 09	0 1f0	0 1f	0 0 f
	4	14	5 8	4 f	1	1 37	0 33	0 10	0 171	0 17f	0 034
			7 4	4 9	13	1 49	0 71	0 7	0 179	0 214	0 031
	4	3	?		1	0 9	0 70	0 17	—0 073	0 037	0 0 0
	4	3 3		4 4	?	1 f0	0 74	0 04	0 16	0 181	0 f ?
M			1 0	0f 0	0f 0	0 9	0 07	0 0	—0 104	0 033	0 0 3
	4	1	8	30	?	1 18	0 f9	0 71	0 012	0 710	0 0 3
		13	9 3	41 1	11 4	1 3	0 37	0 10	0 117	0 11	0 03
	4	4	1	1		1 34	0 59	0 09	0 093	0 18	0 0 7
		13	3	5	4	1 3	0 0	0 07	0 039	0 18	0 017
	44			49	3 4	1 54	0 f?	0 04	0 137	0 1 4	0 014
		74	8 9	0 5	3	1 5	0 7	0 0	0 157	0 1 4	0 013
		33	4	1 9	4	1 44	0 f	0 f	0 119	0 18	0 01f
		74	4		f 1	1 3	0 4	0 0	0 110	0 144	0 01f
	4	4	4	5 0	8 ?	1 30	0 3	0 0	0 09	0 1?	0 0 3
		14	9	4 4	1?	1 15	0 41	0 11	0 01?	0 1 1	0 013
	4		3 0	18 4	13 0	0 1	0 03	0 0	—0 1 1	0 0 ?	0 03
		8	4 1	3 8	1 8	1 44	0 f4	0 0?	0 173	0 1 3	0 0
	4		5	5 9	3 5	0 09	0 33	0 ?7	0 7	0 1 9	0 134
			1	30 4	11 4	1 00	0 17	0 0	0 001	0 0 1	0 0
	4		00	00 0	00 0	0 00	0 00	0 00	0 000	0 000	0 000
		4	14	1 ?	f 1	1 19	0 0	0 ?	0 018	0 1f3	0 0
	4 44		1	3 3	1 8	1 4	0 4	0 1	0 14f	0 1 9	0 0 0
	4 4		8	41	18	1 4	0 41	0 18	0 094	0 134	0 0
	4	1		1?	7 3	0 8	0 33	0 19	0 113	0 181	0 10
		1	14			1 09			0 03		
	4	1	0			1 43			0 1		
	4										
	4		44 8	33 3	f 0	1 73	0 41	0 0	0 0	0 149	0 0

N a z x s l l s s an a l e r f n

Women

N	Cholesterol mg/100 ml			Triglycerides mmole/l			Triglycerides log mmole l		
	$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$
4	238.5	18.9	9.5	0.97	0.14	0.07	—0.018	0.064	0.032
18	207.3	42.6	10.0	0.94	0.26	0.06	—0.045	0.127	0.030
11	226.3	44.3	13.4	0.99	0.31	0.09	—0.025	0.142	0.043
11	272.4	40.1	12.1	1.07	0.58	0.18	—0.079	0.241	0.073
39	279.7	58.5	9.4	1.22	0.53	0.09	0.052	0.171	0.027
107	266.6	56.6	5.5	1.09	0.45	0.04	0.005	0.160	0.016
89	288.2	52.7	5.6	1.24	0.53	0.06	0.057	0.177	0.019
61	295.7	58.2	7.5	1.21	0.38	0.05	0.060	0.133	0.017
27	307.5	53.8	10.3	1.23	0.35	0.07	0.076	0.104	0.020
6	318.8	62.4	25.5	1.24	0.57	0.23	0.056	0.194	0.079
2	255.5	77.1	54.5	1.03	0.45	0.32	—0.011	0.195	0.138
0	—	—	—	—	—	—	—	—	—
375	277.4	58.7	3.0	1.16	0.46	0.02	0.032	0.161	0.008
4	208.5	22.5	11.3	0.84	0.19	0.10	—0.084	0.104	0.052
20	231.3	51.1	11.4	0.99	0.31	0.07	—0.027	0.148	0.033
26	229.8	57.0	11.2	1.01	0.36	0.07	—0.024	0.165	0.032
21	244.0	43.1	9.4	1.10	0.55	0.12	0.000	0.182	0.040
73	259.6	47.0	5.5	1.10	0.63	0.07	0.011	0.146	0.017
161	265.9	52.0	4.1	1.05	0.39	0.03	—0.009	0.175	0.014
119	288.9	55.9	5.1	1.22	0.47	0.04	0.057	0.162	0.015
91	307.9	53.2	5.6	1.32	0.47	0.05	0.097	0.144	0.015
42	310.7	63.8	9.8	1.29	0.68	0.11	0.078	0.154	0.024
12	336.8	48.7	14.1	1.21	0.28	0.08	0.071	0.103	0.030
2	301.5	16.3	11.5	1.10	0.14	0.10	0.039	0.056	0.040
1	492.0	—	—	1.38	—	—	0.139	—	—
72	277.8	59.2	2.5	1.16	0.49	0.02	0.031	0.164	0.007
0	—	—	—	—	—	—	—	—	—
1	161.0	—	—	0.62	—	—	—0.208	—	—
0	—	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—	—
1	280.0	—	—	0.95	—	—	—0.023	—	—
0	—	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—	—
2	220.5	84.1	59.5	0.79	0.23	0.17	—0.015	0.131	0.093

T re f r heal hy men and women group II report ... ars off work.

		c ...	1	d pressure		D.as olic blood pressure mm Hg		
	\			s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$
				—	—	80 0	—	
				3 5	2.5	5 0	1	5.0
				9 4	2 6	80 8	0	1 9
				1 0	2 3	82.4	8	1 6
				1 0	1 5	83 0	8 9	1 1
				1? 9	1 5	84 0	6.5	0.8
				1 4	1 4	84	6 9	0 8
		(		1? 4	1 6	86 1	8	1 0
				11...	1 8	86.1	8.5	1.5
	(			1	3 4	85 4	8 0	2 1
				9 5	3 6	81 4	10.3	3.9
				1 4	6 0	95 0	5 0	2 0
	5			1? 5	0 6	84 4		0 4
				14 1	1 0	90 0	0 0	0.0
	0—			11 1	3 4	81 4	6.0	1 8
	? ?	1		6 5	1 8	6.9	6 6	1 8
	0 34			1? 3	1 8	81 1	2	1 0
	35	1		1? 4	1.2	94 4	8.0	0.8
	40—44	7		13	1 0	85.2	8.3	0 6
	4 —49	18	1	15 8	1 0	86 9	9.3	0 6
	0 54	1	15	15 6	1 3	86	9.1	0
	55 9	84	141 1	14	1 6	87 ?	5	0 8
	60—64	40	1 ?	15	2 5	87.5	8	1 ?
	65— 9	14	1 9 5	14 9	4 0	86 4	6.0	1 6
	0— 4	2	165 0	00 0	0 0	9 5	5 5	2.5
	15— 4	9 6	135 9	14 4	0 5	85 6	8.2	0.3
Hard	15—19	0	—	—	—	—	—	—
	0—24	?	125 0	21.2	15 0	92.5	10 6	5
	25—29		126 4	10 7	4 0	9.3	6 1	2.5
	30—34	0	—	—	—	—	—	—
	35—39	4	126.3	18 9	9 4	3 8	5	?
	40—44	9	1? 2	11.2	3	9 4	11 0	3.
	45—49	5	134 0	10 8	4 8	8 0	9	4 4
	50—54	3	128.3	5 8	3 3	86	6	4 4
	55—59	1	140 0	—	—	90 0	—	—
	60—64	1	120 0	—	—	80 0	—	—
	65—69	0	—	—	—	—	—	—
	0— 4	0	—	—	—	—	—	—
	15— 4	3?	126	12 0	2 1	81 1	9 1	1 6

\ = number of subjects, $\bar{x}$ mean value s standard deviat on $s_{\bar{x}}$ standard error of the mean

Women

N	Systolic blood pressure mm Hg			Diastolic blood press mm Hg		
	$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	
4	121 3	6 3	3 1	76 3	4 8	2 4
18	120 8	5 5	1 3	75 8	4 3	1
11	121.2	16.2	4 9	6 4	10 3	3 1
11	118 2	8 4	2 5	5	4	1 4
39	12[illegible] 4	10 9	1 8	9	5	1
107	1[illegible]8 6	11 2	1 1	81 4	1	7
89	131 5	12 5	1 3	81	7	[illegible]
61	137.2	14 4	1 8	84	8 3	1 [illegible]
27	147 2	14 6	[illegible] 8	83 3	[illegible]	1 4
6	144 2	16 6	6 8	90 8	4	3 0
2	137 5	3 5	2 5	85 0	1	0
0	—	—	—			
375	131 7	13 8	0 7	81 8	8 0	0 4
4	118 8	4 8	2 4	76 3	4 8	[illegible] 4
20	119 0	7 5	1 7	74 3	8 [illegible]	1 8
[illegible]6	1[illegible] 1	10 4	2 0	77 5	8 3	1 6
21	1[illegible] 9	10 1	[illegible] 2	77 9	7 2	1 6
73	128 [illegible]	13 8	1 6	8[illegible] 4	8 0	0 9
161	131 2	15 0	1 [illegible]	8[illegible] 8	8 6	0 7
119	133 6	14 2	1 3	83 7	8 4	0 8
91	139 8	15 1	1 6	85 6	8 7	0 9
4[illegible]	143 6	14 6	[illegible]	89 0	7 9	1 2
12	154 6	18 1	[illegible]	90 8	6 3	1 8
2	160 0	00 0	0 0	[illegible]	[illegible]	2 5
1	170 0	—		95 0		
572	133 0	15 8	0 7	83 3	8 9	0 4
0	—	—				—
1	115 0			85 0		
0	—	—				
0	—	—			—	—
0	—	—				—
1	120 0	—		7[illegible]0		
0	—	—			—	—
0	—	—			—	—
0	—	—	—			—
0	—	—	—			—
0	—	—			—	
0	—	—	—			—
2	117 5	3 5	2 5	80 0	7 1	5 0

Table A 64 Weight height index for healthy men and women (group 10) reporting different degrees of physical activity off work

Physical activity	Age years	Men			
		N	$\bar{x}$ kg/(cm — 100)	s kg/(cm — 100)	$s_{\bar{x}}$ kg/(cm — 100)
None	15—19	1	0.760	—	—
	20—24	2	0.815	0.007	0.0050
	25—29	13	0.932	0.073	0.0203
	30—34	23	0.896	0.109	0.0228
	35—39	62	0.917	0.149	0.0189
	40—44	73	0.958	0.092	0.0108
	45—49	78	0.955	0.088	0.0099
	50—54	61	0.944	0.089	0.0114
	55—59	41	0.940	0.089	0.0139
	60—64	14	1.007	0.092	0.0246
	65—69	7	0.942	0.105	0.0399
	70—74	3	0.910	0.060	0.0351
	15—74	378	0.942	0.104	0.0053
Moderate	15—19	2	0.835	0.021	0.0149
	20—24	11	0.883	0.104	0.0313
	25—29	13	0.851	0.084	0.0234
	30—34	48	0.911	0.080	0.0116
	35—39	113	0.924	0.079	0.0074
	40—44	207	0.936	0.111	0.0077
	45—49	184	0.950	0.085	0.0062
	50—54	138	0.943	0.117	0.0100
	55—59	84	0.952	0.128	0.0140
	60—64	40	0.931	0.112	0.0178
	65—69	14	0.915	0.130	0.0347
	70—74	2	0.915	0.035	0.0250
	15—74	856	0.936	0.104	0.0035
Hard	15—19	0	—	—	—
	20—24	2	0.905	0.063	0.0450
	25—29	7	0.837	0.069	0.0261
	30—34	0	—	—	—
	35—39	4	0.862	0.046	0.0232
	40—44	9	0.917	0.056	0.0187
	45—49	5	0.876	0.089	0.0400
	50—54	3	0.816	0.005	0.0033
	55—59	1	1.020	—	—
	60—64	1	0.810	—	—
	65—69	0	—	—	—
	70—74	0	—	—	—
	15—74	32	0.876	0.072	0.0127

N number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

Women

N	$\bar{x}$ kg (cm — 100	s kg (cm — 100	s_x kg c[illegible]
4	0 910	0 118	0 0[illegible]
18	0 833	0 079	0 01[illegible]
1[illegible]	0 898	0 13[illegible]	0 [illegible]41
11	0 903	0 077	0 [illegible]
39	0 913	0 092	0 0[illegible]
[illegible]0	0 940	0 083	0 0[illegible]
89	0 949	0 084	0 [illegible]
61	0 9[illegible]	0 091	0 [illegible]11
[illegible]	0 972	0 09[illegible]	[illegible]1[illegible]
6	1 0[illegible]3	0 043	0 0[illegible]
[illegible]	1 0[illegible]0	0.212	0 14
0	—	—	—
3[illegible]	0 939	0 093	0 0[illegible]
4	0 810	0 18[illegible]	0 [illegible]
20	0 851	0 033	0 01[illegible]
[illegible]6	0 869	0 106	0 [illegible]
[illegible]	0 939	0 094	0 0[illegible]
3	0 930	0 093	0 [illegible]11[illegible]
161	0 933	0 100	0 0[illegible]
119	0 93[illegible]	0 12[illegible]	0 0114
91	0 960	0 103	0 01[illegible]
4[illegible]	0 9[illegible]8	0 091	0 0140
1[illegible]	0 9[illegible]0	0 129	0 03[illegible]4
[illegible]	0 88[illegible]	0 120	0 0[illegible]49
1	1 030	—	—
5[illegible]	0 934	0 103	0 004[illegible]
4	—	—	—
1	0 840	—	—
0	—	—	—
0	—	—	—
0	—	—	—
1	0.830	—	—
0	—	—	—
0	—	—	—
0	—	—	—
0	—	—	—
0	—	—	—
0	—	—	—
2	0 86[illegible]	0 03[illegible]	0 0[illegible]0

Table A 63 Serum cholesterol and serum triglyceride concentration and log serum triglyceride concentration for healthy men and women (group 10) with different smoking habits

Smoking	Age years	Men N	Cholesterol mg/100 ml $\bar{x}$	s	$s_{\bar{x}}$	Triglycerides mmole/l $\bar{x}$	s	$s_{\bar{x}}$	Triglycerides log mmole/l $\bar{x}$	s	$s_{\bar{x}}$
None	15–19	1	156.0	—	—	0.74	—	—	−0.131	—	—
	20–24	9	210.6	29.2	9.7	1.03	0.43	0.14	−0.027	0.201	0.067
	25–29	15	239.3	50.9	13.1	1.21	0.38	0.10	0.061	0.140	0.036
	30–34	27	241.5	45.4	8.7	1.35	0.55	0.11	0.099	0.162	0.031
	35–39	65	257.5	45.8	5.7	1.33	0.55	0.07	0.089	0.169	0.021
	40–44	99	268.9	46.1	4.6	1.41	0.54	0.05	0.120	0.160	0.016
	45–49	82	284.5	53.2	5.9	1.59	0.64	0.07	0.169	0.167	0.019
	50–54	61	267.2	60.3	7.7	1.33	0.56	0.07	0.088	0.175	0.023
	55–59	31	269.7	55.5	10.0	1.38	0.41	0.07	0.119	0.132	0.024
	60–64	11	279.0	50.2	15.1	1.56	0.28	0.08	0.185	0.078	0.024
	65–69	5	290.2	79.6	35.6	1.29	0.49	0.22	0.079	0.187	0.084
	70–74	2	280.5	4.9	3.5	0.92	0.38	0.27	−0.056	0.185	0.131
	15–74	408	266.2	52.8	2.6	1.40	0.55	0.03	0.113	0.166	0.008
Moderate	15–19	2	151.0	7.1	5.0	0.84	0.01	0.01	−0.079	0.003	0.003
	20–24	6	217.5	38.0	15.5	1.34	0.81	0.33	0.069	0.240	0.098
	25–29	14	246.9	50.6	13.5	1.43	0.61	0.16	0.123	0.168	0.045
	30–34	26	264.9	60.6	11.9	1.46	0.61	0.12	0.129	0.178	0.035
	35–39	77	273.0	59.1	6.7	1.47	0.70	0.08	0.126	0.185	0.021
	40–44	121	283.6	50.0	4.5	1.68	0.85	0.08	0.178	0.194	0.018
	45–49	127	286.0	50.6	4.5	1.61	0.71	0.06	0.171	0.171	0.015
	50–54	98	281.2	56.7	5.7	1.51	0.63	0.06	0.141	0.179	0.018
	55–59	69	285.0	47.0	5.7	1.43	0.55	0.07	0.122	0.171	0.021
	60–64	30	282.7	54.0	9.9	1.22	0.33	0.06	0.071	0.124	0.023
	65–69	13	270.5	39.2	10.9	1.36	0.58	0.16	0.095	0.186	0.052
	70–74	3	245.7	18.6	10.7	0.82	0.06	0.04	0.036	0.031	0.018
	15–74	586	279.1	53.6	2.2	1.52	0.70	0.03	0.143	0.189	0.007
Heavy	15–19	0	—	—	—	—	—	—	—	—	—
	20–24	0	—	—	—	—	—	—	—	—	—
	25–29	1	438.0	—	—	3.47	—	—	0.540	—	—
	30–34	8	220.3	44.9	15.9	1.21	0.35	0.12	0.061	0.160	0.057
	35–39	15	298.1	67.9	17.5	1.75	0.91	0.23	0.203	0.185	0.048
	40–44	30	274.8	46.4	8.5	1.82	0.74	0.13	0.229	0.167	0.031
	45–49	20	294.6	55.0	12.3	1.69	0.68	0.15	0.200	0.152	0.034
	50–54	10	254.8	36.0	11.4	1.54	0.69	0.22	0.133	0.257	0.081
	55–59	10	300.5	38.2	12.1	1.40	0.42	0.13	0.126	0.133	0.042
	60–64	2	284.5	21.9	15.5	1.26	0.62	0.44	0.072	0.223	0.158
	65–69	1	274.0	—	—	0.92	—	—	−0.037	—	—
	70–74	0	—	—	—	—	—	—	—	—	—
	15–74	97	280.5	55.7	5.7	1.66	0.73	0.07	0.182	0.181	0.018

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

Women

N	Cholesterol mg/100 ml			Triglycerides mmole/l			Triglycerides log mmole/l		
	$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	s_x	$\bar{x}$	s	s_x
4	224.8	26.8	13.4	0.79	0.13	0.07	—0.107	0.079	0.040
20	215.5	42.9	10.0	0.98	0.30	0.07	—0.033	0.143	0.032
16	228.6	63.3	15.8	0.94	0.35	0.09	—0.055	0.163	0.041
14	256.3	50.8	13.6	1.09	0.55	0.15	—0.013	0.210	0.056
60	272.5	56.6	7.3	1.04	0.39	0.05	—0.005	0.135	0.018
164	268.0	52.7	4.1	1.03	0.38	0.03	—0.017	0.174	0.014
128	283.0	47.4	4.2	1.10	0.43	0.04	0.012	0.158	0.014
87	297.4	55.0	5.9	1.19	0.40	0.04	0.054	0.134	0.014
45	306.2	62.1	9.3	1.24	0.48	0.07	0.070	0.1[illegible]6	0.019
11	328.5	49.3	14.9	1.20	0.44	0.13	0.052	0.154	0.047
1	310.0	—	—	1.34	—	—	0.127	—	—
1	492.0	—	—	1.38	—	—	0.139	—	—
551	277.7	57.4	2.4	1.09	0.41	0.02	0.009	0.158	0.007
3	225.7	32.3	18.7	1.08	0.03	0.02	0.033	0.013	0.008
18	218.4	54.6	12.9	0.93	0.28	0.07	—0.049	0.139	0.033
17	232.9	41.8	10.1	1.02	0.32	0.08	—0.016	0.150	0.036
17	249.9	38.9	9.4	1.12	0.58	0.14	0.002	0.200	0.049
44	260.7	42.5	6.4	1.16	0.37	0.06	0.043	0.139	0.021
91	259.8	54.3	5.7	1.13	0.47	0.05	0.021	0.159	0.017
69	295.6	64.0	7.7	1.45	0.51	0.06	0.135	0.148	0.018
52	310.8	59.1	8.2	1.39	0.46	0.06	0.119	0.143	0.020
19	*318.4*	*57.0*	*13.1*	*1.37*	*0.79*	*0.18*	*0.098*	*0.164*	*0.038*
7	334.6	61.0	23.1	1.26	0.31	0.12	0.089	0.103	0.039
1	313.0	—	—	1.20	—	—	0.079	—	—
0	—	—	—	—	—	—	—	—	—
338	275.7	61.6	3.4	1.24	0.50	0.03	0.062	0.160	0.087
0	—	—	—	—	—	—	—	—	—
1	279.0	—	—	0.96	—	—	—0.018	—	—
2	248.5	43.1	30.5	1.40	0.23	0.17	0.141	0.072	0.052
1	284.0	—	—	0.66	—	—	—0.181	—	—
2	229.5	20.5	14.5	1.11	0.30	0.22	0.035	0.121	0.086
5	312.0	65.6	29.4	1.32	0.22	0.10	0.115	0.073	0.033
3	330.3	36.9	21.3	1.73	0.37	0.21	0.231	0.097	0.056
2	300.5	10.6	7.5	1.69	0.45	0.32	0.219	0.117	0.083
0	—	—	—	—	—	—	—	—	—
1	201.0	—	—	0.71	—	—	—0.149	—	—
0	—	—	—	—	—	—	—	—	—
17	286.6	55.1	13.4	1.32	0.40	0.10	0.100	0.141	0.034

TABLE N 66 Systolic and diastolic blood pressure for healthy men and women (group 10) with different smoking habits

Smoking	Age years	Men						
		N	Systolic blood pressure mm Hg			Diastolic blood pressure mm Hg		
			$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$
None	15—19	1	150 0	—	—	90 0	—	—
	20—24	9	131 1	11 7	3 9	82 2	7 9	2 6
	25—29	15	129 7	8 8	2 3	78 3	6 5	1 7
	30—34	27	134 3	12 5	2 4	82 6	8 2	1 6
	35—39	65	131 2	12 5	1 5	84 2	8 5	1 1
	40—44	99	134 1	14 7	1 5	84 0	7 9	0 8
	45—49	82	135 4	13 0	1 4	85 8	7 7	0 8
	50—54	61	137 2	15 4	2 0	87 0	7 7	1 0
	55—59	31	138 4	13 2	2 4	85 5	7 6	1 4
	60—64	11	142 7	13 8	4 2	87 3	4 7	1 4
	65—69	5	139 0	19 5	8 7	85 0	12 2	5 5
	70—74	2	167 5	3 5	2 5	92 5	10 6	7 5
	15—74	408	135 0	14 0	0 7	84 8	8 0	0 4
Moderate	15—19	2	125 0	7 1	5 0	85 0	7 1	5 0
	20—24	6	127 5	11 3	4 6	78 3	2 6	1 1
	25—29	14	132 1	9 6	2 6	80 7	7 3	2 0
	30—34	26	127 3	11 0	2 2	80 6	6 5	1 3
	35—39	77	130 8	12 7	1 4	83 6	8 2	0 9
	40—44	121	133 7	13 7	1 2	84 5	8 1	0 7
	45—49	127	134 8	13 9	1 2	85 6	8 0	0 7
	50—54	98	136 4	14 1	1 4	85 9	8 2	0 8
	55—59	69	139 9	13 1	1 6	86 7	7 5	0 9
	60—64	30	139 2	13 1	2 4	86 0	8 3	1 5
	65—69	13	138 1	11 8	3 3	83 8	6 2	1 7
	70—74	3	160 0	8 7	5 0	91 7	2 9	1 7
	15—74	586	134 8	13 7	0 6	84 9	8 0	0 3
Heavy	15—19	0	—	—	—	—	—	—
	20—24	0	—	—	—	—	—	—
	25—29	1	130 0	—	—	80 0	—	—
	30—34	8	125 0	14 1	5 0	82 5	4 6	1 6
	35—39	15	132 3	15 2	3 9	84 7	10 8	2 8
	40—44	30	136 0	11 7	2 1	86 3	7 6	1 4
	45—49	20	139 8	12 2	2 7	88 8	8 1	1 8
	50—54	10	139 0	15 4	4 9	88 0	9 2	2 9
	55—59	10	146 0	17 8	5 6	93 5	7 8	2 5
	60—64	2	152 5	24 7	17 5	90 0	—	—
	65—69	1	145 0	—	—	90 0	—	—
	70—74	0	—	—	—	—	—	—
	15—74	97	137 0	14 5	1 5	87 2	8 4	0 9

N = number of subjects $\bar{x}$ = mean value, s = standard deviation $s_{\bar{x}}$ = standard error of the mean

Women

N	Systolic blood pressure mm Hg			Diastolic blood pressure mm Hg		
	$\bar{x}$	s	$s_{\bar{x}}$	$\bar{x}$	s	$s_{\bar{x}}$
4	117.5	2.9	1.4	77.5	5.0	2.5
20	120.5	6.3	1.4	73.3	8.0	1.8
16	123.1	10.5	2.6	76.6	9.1	2.3
14	123.2	7.5	2.0	76.8	4.2	1.1
60	128.3	12.8	1.7	81.8	6.9	0.9
164	132.0	13.7	1.1	83.0	8.1	0.6
128	132.8	13.4	1.2	82.8	8.2	0.7
87	139.5	14.0	1.5	85.5	8.4	0.9
45	146.2	12.8	1.9	89.2	7.1	1.1
11	145.9	20.5	6.2	90.5	7.9	2.4
1	140.0	—	—	90.0	—	—
1	170.0	—	—	95.0	—	—
551	133.1	14.3	0.6	83.2	8.5	0.4
3	125.0	5.0	2.9	75.0	5.0	2.9
18	118.9	7.2	1.7	77.2	4.3	1.0
17	121.8	13.5	3.3	77.4	8.3	2.0
17	119.7	11.4	2.8	77.6	7.9	1.9
44	126.5	13.5	2.0	81.0	9.2	1.4
91	126.9	13.6	1.4	80.9	8.3	0.9
69	132.8	14.1	1.7	82.8	7.3	0.9
52	138.8	16.1	2.2	85.9	8.4	1.2
19	142.1	17.5	4.0	88.9	9.1	2.1
7	159.3	8.9	3.4	91.4	3.8	1.4
1	160.0	—	—	95.0	—	—
0	—	—	—	—	—	—
338	130.4	15.8	0.9	82.2	8.6	0.5
0	—	—	—	—	—	—
1	120.0	—	—	80.0	—	—
2	122.5	24.7	17.5	82.5	17.7	12.5
1	120.0	—	—	70.0	—	—
2	130.0	14.1	10.0	75.0	7.1	5.0
5	127.0	10.4	4.6	80.0	7.1	3.2
3	126.7	16.1	9.3	78.3	14.4	8.3
2	137.5	3.5	2.5	85.0	7.1	5.0
0	—	—	—	—	—	—
0	—	—	—	—	—	—
1	135.0	—	—	80.0	—	—
0	—	—	—	—	—	—
17	127.6	11.7	2.8	79.4	8.8	2.1

Table A 67 Weight height index for healthy men and women (group 10) with different smoking habits

Smoking	Age years	Men			
		N	$\bar{x}$ kg/(cm — 100)	s kg/(cm — 100)	$s_{\bar{x}}$ kg/(cm — 100)
None	15—19	1	0.850	—	—
	20—24	9	0.848	0.097	0.0325
	25—29	15	0.896	0.074	0.0191
	30—34	27	0.906	0.089	0.0171
	35—39	65	0.931	0.090	0.0112
	40—44	99	0.938	0.130	0.0131
	45—49	82	0.973	0.090	0.0099
	50—54	61	0.930	0.140	0.0180
	55—59	31	0.934	0.182	0.0328
	60—64	11	0.967	0.108	0.0325
	65—69	5	0.972	0.174	0.0780
	70—74	2	0.915	0.035	0.0250
	15—74	408	0.938	0.120	0.0059
Moderate	15—19	2	0.790	0.042	0.0300
	20—24	6	0.920	0.074	0.0302
	25—29	14	0.850	0.100	0.0267
	30—34	26	0.908	0.090	0.0176
	35—39	77	0.909	0.124	0.0141
	40—44	121	0.942	0.092	0.0084
	45—49	127	0.934	0.083	0.0073
	50—54	98	0.937	0.099	0.0100
	55—59	69	0.954	0.088	0.0106
	60—64	30	0.930	0.111	0.0203
	65—69	13	0.917	0.111	0.0203
	70—74	3	0.910	0.060	0.0351
	15—74	586	0.931	0.098	0.0040
Heavy	15—19	0	—	—	—
	20—24	0	—	—	—
	25—29	1	0.980	—	—
	30—34	8	0.870	0.100	0.0355
	35—39	15	0.909	0.123	0.0318
	40—44	30	0.959	0.099	0.0180
	45—49	20	0.944	0.078	0.0174
	50—54	10	0.965	0.076	0.0241
	55—59	10	0.922	0.088	0.0280
	60—64	2	0.775	0.049	0.0350
	65—69	1	0.760	—	—
	70—74	0	—	—	—
	15—74	97	0.932	0.100	0.0101

N = number of subjects $\bar{x}$ = mean value s = standard deviation $s_{\bar{x}}$ = standard error of the mean

Women			
N	$\bar{x}$ kg/(cm — 100)	s kg/(cm — 100)	$s_{\bar{x}}$ kg/(cm — 100)
4	0 902	0 155	0 0778
20	0 866	0 091	0 0204
16	0 864	0 094	0 0235
14	0 979	0 078	0 0210
60	0 934	0 098	0 0127
164	0 936	0 089	0 0069
128	0 949	0 115	0 0102
87	0 964	0 092	0 0099
45	0 962	0 097	0 0145
11	0 993	0 139	0 0419
1	1 200	—	—
1	1 030	—	—
551	0 943	0 102	0 0043
3	0 800	0 193	0 1115
18	0 828	0 065	0 0154
17	0 870	0 126	0 0307
17	0 884	0 078	0 0189
44	0 911	0 096	0 0145
91	0 930	0 100	0 0105
69	0 921	0 087	0 0104
52	0 953	0 108	0 0150
19	0 974	0 087	0 0201
7	1 005	0 067	0 0254
1	0 800	—	—
0	—	—	—
338	0 921	0 102	0 0055
0	—	—	—
1	0 830	—	—
2	1 050	0 070	0 0500
1	0 920	—	—
2	0 890	0 056	0 0400
5	0 990	0 158	0 0709
3	0 876	0 168	0 0970
2	0 910	0 014	0 0100
0	—	—	—
0	—	—	—
1	0 900	—	—
0	—	—	—
17	0 937	0 121	0 0294

TABLE A 68 Partial correlation coefficients (age constant) for cholesterol on triglycerides and for cholesterol on log triglycerides for men and women in group 10

Age	Men			Women		
years	N	Cholesterol—triglycerides	Cholesterol—log triglycerides	N	Cholesterol—triglycerides	Cholesterol—log triglycerides
15—19	3	—0.000	0.000	8	0.451	0.505
20—24	15	0.175	0.065	39	0.361*	0.387*
25—29	33	0.659***	0.565***	37	0.25[illegible]	0.263
30—34	71	0.469***	0.479***	32	—0.087	—0.036
35—39	179	0.278***	0.309***	112	0.244**	0.229*
40—44	289	0.419***	0.393***	269	0.223***	0.248***
45—49	267	0.277***	0.320***	208	0.349***	0.320***
50—54	207	0.393***	0.397***	152	0.166*	0.166*
55—59	126	0.138	0.139	69	—0.047	0.033
60—64	55	0.093	0.102	18	—0.051	—0.007
65—69	21	0.649**	0.671***	4	0.942	0.973*
70—74	5	0.700	0.603	1	0.000	0.000
15—74	1266	0.358*	0.366***	949	0.209***	0.229***

TABLE A 69 Partial correlation coefficients (age constant) for serum cholesterol and serum triglyceride concentration and for log serum triglyceride concentration on systolic and diastolic blood pressure for men in group 10

Age years	N	Cholesterol—systolic blood pressure	Triglycerides—systolic blood pressure	log Triglycerides—systolic blood pressure	Cholesterol—diastolic blood pressure	Triglycerides—diastolic blood pressure	log Triglycerides—diastolic blood pressure
15—19	3	0.000	—0.999	—0.999	1.250	—0.000	—0.000
20—24	15	0.016	0.148	0.214	—0.138	—0.271	—0.279
25—29	33	—0.058	—0.010	—0.093	—0.007	—0.0[illegible]	—0.041
30—34	71	—0.015	—0.002	0.031	0.035	0.007	0.002
35—39	179	0.153	0.067	0.086	0.201**	0.097	0.120
40—44	289	0.105	0.105	0.103	0.125	0.075	0.085
45—49	267	0.091	0.064	0.077	0.141*	0.011	0.070
50—54	207	—0.110	—0.073	0.009	—0.015	—0.093	—0.0[illegible]
55—59	126	0.118	—0.001	0.038	0.084	0.010	0.044
60—64	55	—0.136	0.201	0.189	—0.150	0.175	0.150
65—69	21	0.48	0.438	0.507	0.211	0.425	0.432
70—74	5	0.533	—0.1[illegible]	—0.308	0.517	0.930*	0.961
15—74	1[illegible]	0.041	0.050	0.065	0.093*	0.045	0.063*

TABLE A [illegible]0 Partial correlation coefficients (age constant) for serum-cholesterol and serum triglyceride concentration and for log serum triglyceride concentration [illegible] and diastolic blood pressure for women in group 10

Age years	N	Cholesterol—systolic blood pressure	Triglycerides—systolic blood pressure	log Triglycerides—systolic blood pressure	Cholesterol—diastolic blood pressure	Triglycerides—diastolic blood pressure	log Triglycerides—diastolic blood pressure
15—19	8	0.191	0.774	0.741*	—0.[illegible]	—0.313	—0[illegible]
20—24	39	0.181	0.4[illegible]4*	0.45[illegible]**	0.221	0.0[illegible]	[illegible].09[illegible]
25—29	37	0.163	0.149	0.131	0.234	0.201	0.18
30—34	37	—0.100	0.165	0.104	—0.090	0.220	0.[illegible]61
35—39	117	0.023	0.051	0.079	—0.031	—0.030	0.0[illegible]3
40—44	269	0.159	—0.014	0.079	0.175	—0.071	[illegible].01
45—49	208	—0.015	0.101	0.096	0.023	0.169	0.15[illegible]
50—54	157	—0.013	0.056	0.058	—0.075	0.106	0.12
55—59	69	0.035	0.126	0.209	0.204	0.058	0.186
60—64	18	0.417	—0.115	—0.086	0.3[illegible]0	—0.4[illegible]3	—0.418
65—69	4	0.973*	0.840	0.895	0.991**	0.9[illegible]*	0.994
70—74	1	0.000	0.000	0.000	0.000	0.000	0.000
15—74	949	0.068*	0.054	0.0[illegible]0*	0.085**	0.056	0.078

TABLE A 71 Partial correlation coefficients (*age constant*) *for serum cholesterol* and serum triglyceride concentration and for log serum triglyceride concentration on weight height index for men and women in group 10

Age years	Men				Women			
	N	Cholesterol—weight/height index	Triglycerides weight/height index	log Triglycerides—weight/height index	N	Cholesterol—weight/height index	Triglycerides—weight/height index	log Triglycerides—weight/height index
15—19	3	0.000	−0.999*	−0.999*	8	0.591	0.051	0.085
20—24	15	−0.053	0.193	0.193	39	0.053	−0.163	−0.212
25—29	33	0.149	0.353*	0.376	37	0.228	0.190	0.195
30—34	71	0.1[illegible]	0.340**	0.348**	32	0.084	0.218	0.184
35—39	179	0.044	0.173*	0.194**	112	0.071	0.066	0.056
40—44	289	0.166*	0.248***	0.283***	269	0.024	0.123*	0.151*
45—49	267	0.115	0.258***	0.269***	208	0.080	0.054	0.037
50—54	202	0.033	0.173*	0.177*	152	−0.039	0.110	0.105
55—59	126	0.206*	0.171	0.166	69	0.004	0.088	0.157
60—64	55	−0.002	0.126	0.097	18	−0.019	−0.334	−0.339
65—69	21	0.599***	0.419	0.172*	4	−0.749	−0.927	−0.880
70—74	5	0.561	0.576	0.582	1	0.000	0.000	0.000
15—74	1266	0.130***	0.231***	0.246***	949	0.033	0.095**	0.088

Table 5.72 Partial correlation coefficients (age constant) for weight/height index on systolic and diastolic blood pressure for men and women in group 10

Age years	Men			Women		
	N	Weight/height index systolic blood pressure	Weight/height index diastolic blood pressure	N	Weight/height index systolic blood pressure	Weight/height index—diastolic blood pressure
1 19	3	0 999*	0 000	8	0 0 2	0 002
20 24	1	0 402	0 92	39	101	0 077
25 29	33	[illegible] 5	0 143	37	0 393*	0 221
30 34	71	0 248*	0 236*	32	0 401*	0 41 *
35 39	173	0 181*	0 138	112	21 **	0 218*
40—44	89	0 25 ***	0 233***	263	0 2 2***	[illegible] 16***
45—49	2 7	0 195**	0 219***	209	0 [illegible]***	[illegible]28 ***
50—54	202	0 271***	0 25 ***	1	0 1 9***	[illegible]31[illegible]***
55—59	12	0 031	0 0 3	[illegible]	0 1 3	[illegible] 111
60—64	[illegible]	0 47	0 310	18	0 11	[illegible] 131
65—69	21	0 10 **	[illegible] 3 3	4	7	
70—74		0 172	0 713	1	[illegible]	
15—74	12	0 211***	1 203***	19	0***	*

Table A 77 Cholesterol concentration in different months for healthy men (group 10)

	Men											
	January		February		March		April		May		June	
Age years	N	$\bar{x}$ mg/100 ml	N	$\bar{x}$ mg/100 ml	N	$\bar{x}$ mg/100 ml	N	$\bar{x}$ mg/100 ml	N	$\bar{x}$ mg/100 ml	N	$\bar{x}$ mg/100 ml
15—19	2	156.0	0	—	0	—	0	—	0	—	0	—
20—24	1	218.0	1	183.0	3	238.3	1	170.0	0	000.0	1	219.0
25—29	2	277.0	5	220.6	4	289.5	2	276.5	4	215.5	5	231.0
30—34	2	189.0	10	242.7	9	249.4	6	226.8	9	291.1	5	246.4
35—39	15	276.1	14	250.4	26	283.7	16	301.0	19	259.3	14	263.0
40—44	24	275.4	31	274.9	18	294.1	21	257.3	27	272.2	24	270.4
45—49	29	292.9	18	267.0	29	275.3	29	289.1	18	270.8	9	302.1
50—54	19	282.5	24	262.3	17	277.3	22	272.5	15	294.9	15	250.5
55—59	9	280.4	11	276.9	16	286.8	12	286.3	6	289.7	10	245.9
60—64	9	308.7	6	249.5	6	258.0	3	282.0	3	244.3	6	270.8
65—69	1	328.0	2	326.0	5	278.0	1	237.0	1	288.0	1	299.0
70—74	0	—	3	262.0	1	251.0	0	—	0	—	0	—
15—74	113	280.6	125	262.6	134	278.1	113	276.1	102	272.7	90	262.7

	July		August		September		October		November		December	
Age years	N	$\bar{x}$ mg/100 ml	N	$\bar{x}$ mg/100 ml	N	$\bar{x}$ mg/100 ml	N	$\bar{x}$ mg/100 ml	N	$\bar{x}$ mg/100 ml	N	$\bar{x}$ mg/100 ml
15—19	1	146.0	0	—	0	—	0	—	0	—	0	—
20—24	3	216.7	2	193.0	0	—	1	218.0	1	177.0	1	264.0
25—29	4	202.3	5	250.6	0	—	0	000.0	2	331.5	0	000.0
30—34	6	226.8	7	268.1	5	214.4	5	267.0	3	237.7	4	238.3
35—39	13	230.7	10	276.3	13	260.2	11	254.2	19	271.2	9	277.9
40—44	15	269.5	20	291.6	23	301.5	23	294.3	29	265.9	34	264.3
45—49	6	293.3	21	271.1	25	277.1	32	281.6	26	309.7	25	303.4
50—54	9	243.6	10	262.2	21	277.2	16	304.7	15	292.1	19	262.0
55—59	*3*	*292.3*	*7*	*275.7*	*6*	*269.2*	*13*	*281.4*	*19*	*281.9*	*13*	*301.5*
60—64	2	275.0	3	311.3	8	285.0	1	318.0	4	281.5	4	264.8
65—69	1	199.0	0	—	3	248.7	3	311.0	2	233.5	1	210.0
70—74	1	261.0	0	—	0	—	0	—	0	—	0	—
15—74	64	247.6	85	274.0	104	277.9	105	285.3	120	281.7	110	276.9

N = number of subjects $\bar{x}$ — mean value

TABLE A 8 Cholesterol concentration in different months for healthy women (group 10)

Women

Age years	January N	January $\bar{x}$ mg/100 ml	February N	February $\bar{x}$ mg/100 ml	March N	March $\bar{x}$ mg/100 ml	April N	April $\bar{x}$ mg/100 ml	May N	May $\bar{x}$ mg/100 ml	June N	June $\bar{x}$ mg/100 ml
15—19	1	227.0	1	234.0	1	250.0	0	—	0	—	0	—
20—24	1	176.0	2	200.5	8	184.3	5	218.[illegible]	1	327.0	4	248.5
25—29	3	256.3	3	183.7	5	242.0	3	238.7	2	313.5	2	171.5
30—34	5	264.8	0	—	1	179.0	2	318.5	3	249.3	4	251.0
35—39	6	285.5	7	247.9	12	301.3	11	264.0	16	254.[illegible]	4	239.0
40—44	17	262.7	22	263.6	29	259.7	19	256.1	30	260.8	25	260.8
45—49	9	316.1	29	286.6	29	292.0	12	303.2	27	283.3	19	282.1
50—54	11	280.2	18	285.9	22	302.6	8	291.0	21	313.3	14	307.3
55—59	3	311.3	6	318.3	8	277.4	6	314.8	5	313.0	9	311.4
60—64	1	429.0	0	—	2	348.5	4	346.0	1	281.0	1	337.0
65—69	0	—	0	—	0	—	0	—	0	—	0	—
70—74	0	—	0	—	0	—	0	—	0	—	0	—
15—74	57	280.1	88	273.9	117	276.1	70	278.2	10[illegible]	280.0	8[illegible]	275.8

Age years	July N	July $\bar{x}$ mg/100 ml	August N	August $\bar{x}$ mg/100 ml	September N	September $\bar{x}$ mg/100 ml	October N	October $\bar{x}$ mg/100 ml	November N	November $\bar{x}$ mg/100 ml	December N	December $\bar{x}$ mg/100 ml
15—19	0	—	0	—	3	202.3	1	238.0	0	—	1	212.[illegible]
20—24	3	178.0	6	265.7	5	218.2	2	211.0	0	—	2	[illegible]
25—29	3	184.0	7	231.0	4	227.3	2	224.[illegible]	2	2[illegible].0	1	27[illegible]
30—34	4	263.3	4	270.[illegible]	3	237.7	3	259.7	3	2[illegible]	[illegible]	[illegible]
35—39	10	241.1	5	252.4	13	264.2	9	260.[illegible]	13	27[illegible]	7	[illegible]
40—44	24	268.6	17	260.9	13	283.7	34	272.7	17	27[illegible]	22	[illegible]
45—49	12	249.6	15	289.5	12	292.9	18	302.0	14	2[illegible].1	12	227
50—54	14	294.7	7	343.4	11	309.3	10	312.3	10	[illegible]	7	[illegible]
55—59	7	269.4	2	292.0	2	328.0	8	32[illegible]	8	[illegible]	[illegible]	[illegible]
60—64	1	334.0	1	301.0	2	328.0	1	271.0	4	[illegible]	[illegible]	[illegible]
65—69	0	—	1	201.0	1	290.0	1	310.0	1	[illegible]	0	—
70—74	0	—	0	—	0	—	0	—	1	4[illegible]	[illegible]	[illegible]
15—74	78	260.7	65	274.2	69	274.[illegible]	8[illegible]	27[illegible].1	7[illegible]	2[illegible]	[illegible]	27[illegible]

N = number of subjects $\bar{x}$ — mean value

TABLE A 79 Triglyceride concentration in different months for healthy men (group 10

	Men											
	January		February		March		April		May		June	
Age years	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l
15—19	2	0.79	0	—	0	—	0	—	0	—	0	—
20—24	1	1.40	1	1.89	3	0.85	1	0.68	0	—	1	0.84
25—29	2	1.94	5	1.04	4	1.34	2	1.76	4	1.21	5	1.17
30—34	2	0.97	10	1.53	9	1.52	6	1.27	9	1.41	5	1.19
35—39	15	1.47	14	1.26	26	1.48	16	1.29	19	1.43	14	1.73
40—44	24	1.59	31	1.79	18	1.81	21	1.27	27	1.42	24	1.56
45—49	29	1.52	18	1.77	29	1.74	29	1.45	18	1.74	9	1.73
50—54	19	1.48	24	1.39	17	1.41	22	1.48	15	1.64	15	1.47
55—59	9	1.27	11	1.64	16	1.49	12	1.52	6	1.26	10	1.34
60—64	9	1.21	6	1.30	6	1.33	3	1.63	3	1.27	6	1.42
65—69	1	1.50	2	1.97	5	1.24	1	1.11	1	1.90	1	1.20
70—74	0	—	3	0.91	1	0.77	0	—	0	—	0	—
15—74	113	1.46	125	1.55	134	1.54	113	1.40	102	1.49	90	1.50

	July		August		September		October		November		December	
Age years	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l
15—19	1	0.83	0	—	0	—	0	—	0	—	0	—
20—24	3	1.11	2	0.73	0	—	1	1.12	1	1.17	1	2.81
25—29	4	1.46	5	1.34	0	—	0	—	2	2.35	0	—
30—34	6	1.07	7	1.84	5	1.05	5	1.14	3	1.27	4	1.30
35—39	13	1.47	10	1.48	13	1.18	11	1.56	19	1.50	9	1.34
40—44	15	1.48	20	1.61	23	1.67	23	1.59	29	1.51	34	1.57
45—49	6	1.55	21	1.29	25	1.25	32	1.24	26	1.83	25	2.12
50—54	9	1.39	10	1.13	21	1.45	16	1.49	15	1.61	19	1.64
55—59	3	1.51	7	1.18	6	1.37	13	1.52	19	1.43	13	1.37
60—64	2	1.46	3	1.29	8	1.13	1	1.47	4	1.32	4	1.50
65—69	1	0.64	0	—	3	0.93	3	1.50	2	0.89	1	0.94
70—74	1	[illegible].81	0	—	0	—	0	—	0	—	0	—
15—74	64	1.38	85	1.40	104	1.35	105	1.43	120	1.57	110	1.66

N = number of subjects $\bar{x}$ = mean value

TABLE A 80 Triglyceride concentration in different months for healthy women (group 10

Age years	Women											
	January		February		March		April		May		June	
	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l
15—19	1	0.81	1	0.86	1	1.06	0	—	0	—	0	
20—24	1	0.81	2	0.66	8	0.85	5	0.94	1	0.87	4	1.1[illegible]
25—29	3	1.24	3	0.82	5	0.90	3	1.11	2	1.19	2	1.40
30—34	5	1.25	0	—	1	1.20	2	1.03	3	0.68	4	1.[illegible]2
35—39	6	1.17	7	1.23	12	1.45	11	1.09	16	1.03	4	1.06
40—44	17	0.99	22	1.09	29	1.15	19	1.18	30	1.02	25	1.14
45—49	9	1.52	29	1.31	29	1.25	12	1.21	27	1.20	19	1.[illegible]3
50—54	11	1.37	18	1.35	22	1.23	8	1.23	21	1.33	14	1.30
55—59	3	1.97	6	1.01	8	1.23	6	1.05	5	1.18	9	1.10
60—64	1	1.01	0	—	2	1.81	4	1.35	1	1.00	1	0.95
65—69	0	—	0	—	0	—	0	—	0	—	0	—
70—74	0	—	0	—	0	—	0	—	0	—	0	—
15—74	57	1.25	88	1.20	117	1.21	70	1.15	106	1.13	8[illegible]	1.20

Age years	July		August		September		October		November		December	
	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l	N	$\bar{x}$ mmole/l
15—19	0	—	0	—	3	0.85	1	1.12	0	—	1	0.83
20—24	3	1.08	6	0.95	5	0.88	2	1.19	0	—	2	1.26
25—29	3	0.81	7	0.91	4	1.04	2	0.77	2	1.34	1	0.85
30—34	4	1.07	4	0.85	3	1.36	3	0.63	3	1.23	0	—
35—39	10	1.12	5	1.33	13	1.07	9	0.97	13	1.16	7	1.15
40—44	24	0.98	17	0.89	13	1.04	34	0.95	17	1.19	22	1.20
45—49	12	0.93	15	1.28	12	1.03	18	1.25	14	1.15	12	1.34
50—54	14	1.28	7	1.30	11	1.17	10	1.12	10	1.27	7	1.22
55—59	7	1.23	2	1.17	2	0.95	8	1.36	8	1.29	5	1.90
60—64	1	1.32	1	0.63	2	0.90	1	1.16	4	1.28	0	—
65—69	0	—	1	0.71	1	1.00	1	1.34	1	1.20	0	—
70—74	0	—	0	—	0	—	0	—	1	1.38	0	—
15—74	78	1.07	65	1.07	69	1.05	89	1.07	73	1.21	57	1.28

N = number of subjects $\bar{x}$ = mean value

Acta Medica Scandinavica

Supplementum 494

Hematological Investigations in Ethiopia

with special reference to a high iron intake

By Yngve Hofvande

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv*, was founded in 1869 by Professor Axel Key, MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German Acta Medica Scandinavica is published on a non-profit basis

[illegible]scriptions

[illegible] Medica Scandinavica (two [illegible] of six numbers each a year) [illegible] free supplements to the current volumes During the past few years an average number of 17 supplements has been issued per annum

Subscription Rates

Per annum = two volumes
In Denmark Finland, Iceland Norway, Sweden and the Netherlands Sw kr 120, incl postage
Other countries Sw kr 140 incl postage

Chief Editor

Associate Professor Birger Strandell, MD
Birger Jarlsgatan 9
S 111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Biblioteksgatan 11
S-111 46 Stockholm, Sweden

Subscription and Distribution

Almqvist & Wiksell
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1, Sweden

Printers

Almqvist & Wiksells Boktryckeri AB
Box 616
S 751 25 Uppsala 1, Sweden

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv*, was founded in 1869 by Professor Axel Key, MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German Acta Medica Scandinavica is published on a non profit basis

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each a year) ...de free supplements to the cur ...volumes During the past few ... an average number of 17 supplements has been issued per annum

Subscription Rates

Per annum two volumes
In Denmark, Finland Iceland Norway Sweden and the Netherlands Sw kr 120, incl postage
Other countries Sw kr 140 incl postage

Chief Editor

Associate Professor Birger Strandell, MD
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S 111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Biblioteksgatan 11
S-111 46 Stockholm, Sweden

Subscription and Distribution

Almqvist & Wiksell
Gamla Brogatan 26, Box 62
S 101 20 Stockholm 1, Sweden

Printers

Almqvist & Wiksells Boktryckeri AB
Box 616
S 751 25 Uppsala 1, Sweden

to Ruth Marie

Contents

Acknowledgements

These investigations were carried out at the Children s Nutrition Unit in Addis Ababa in 1965—1967 at the suggestion of Professor Bo Vahlquist head of the Pediatric Department of the University Hospital, Uppsala, and chairman of the Scientific Advisory Group to the CNU project I am greatly indebted to him for his constant interest in my work, for his valuable advice and positive criticism and for arranging excellent working conditions during the compilation of this study

I am also indebted to the other members of the CNU Advisory Group Professors Gunnar Ågren and Olle Mellander and Assistant Professor Gunnar Almgård for valuable discussions and suggestions

The considerable amount of data in this investigation could not have been collected without the coordinated efforts of many CNU s aff members, to whom I am very grateful I am especially indebted to Dr G Tornell who participated in the Ijaji campaign in 1965 and to Dr R Eksmyr who succeeded him and who initiated and organized the anthropometric and clinical studies in privileged children , as well as to Miss T Svensson S R N who skilfully and meticulously organized the blood taking in most campaigns I would also like to express my appreciation to Miss I von Dewall R N and to Ato Chimsa Debissa who patiently and efficintly kept the work in Ijaji at a high standard Community nurse Mullu Melles assisted in the bone marrow study, for which I am very grateful

This study would not have been possible without the skilful technical laboratory assistance provided by B Zaar laboratory engineer, who together with Ato Petros Gabre B Sc and assistants took care of large numbers of samples and efficiently operated the biochemical laboratory, for which I am greatly indebted My thanks are also due to Mrs R Gibson, M Sc , who kindly performed the iron analysis on soil and tef, and to Mr E Kopp who performed the tef cleaning I am further indebted to Assistant Professor K-E Knutsson, Ph D , who succeeded me at CNU for valuable help during the fall 1967 when these studies were completed, and to Professor K. B Bjornesjo and Assistant Professor K Jacobsson for valuable assistance and helpful discussions

I owe special thanks to Assistant Professor A Weinfeld Gothenburg who very kindly and generously had the liver iron analysis performed under his supervision

For helpful and rewarding discussions during the compilation of this work I am indebted to Assistant Professor T Mellbin who organized and performed the CNU field surveys during the years 1962—1965 and to Miss R Selinus M Sc who organized and performed the dietary surveys in 1963—1967

I am very grateful to Assistant Professors L Garby A Killander and L Wranne for reviewing the manuscript and for valuable discussions and positive criticism

My thanks are due to Mr Adam Taube lecturer in statistics for his kind advice on certain statistical matters and to Mrs Maud Marsden for revising the English text

I am also grateful to Mrs Birgit Haggqvist who made the ink-drawings and to Miss Kerstin Sjoberg who typed the manuscript

The investigations were performed within the framework of the activities of the Children s Nutrition Unit the budget of which comes mainly from the Swedish International Development Authority (SIDA) I gratefully acknowledge SIDA s support especially in providing excellent working conditions during the compilatory work

Preface

Aim of the present study

In 1958, the Interdepartmental Committee on Nutrition for National Defense (61) made a country wide comprehensive dietary and clinical survey in Ethiopia One of the very interesting discoveries during this study was the enormous dietary intake of iron, amounting to several hundred mg daily in the Ethiopian highlands where the cereal tef constitutes the staple grain It was concluded that iron deficiency anemia was no serious problem and low hemoglobin values were recorded in only one place (Gambella) which had a high frequency of parasitism and was situated at a low altitude The high dietary iron intake has later been confirmed by others (101) In view of the comparatively high altitude at which these investigations were performed the mean Hb values obtained in these studies, especially in adults, would be considered rather low suggesting a suboptimal material Additional studies were therefore considered desirable It also seemed of interest to determine whether the often excessive dietary intake of iron led to a saturation of the iron stores to an extent similar to what has been described in the Bantus

The aim of this investigation was thus to study

1 the sources of the food iron in the tef diet on the Ethiopian highlands,
2 certain hematological values (Hb PCV, ESR, SI and TIBC) in healthy and apparently healthy subjects of different ages with a high iron intake at a medium high altitude and to try to obtain a reference standard and to estimate the prevalence of anemia among these subjects,
3 the iron stores in various age groups by examination of bone marrow hemosiderin, by desferrioxamine tests and by chemical liver iron determination on an autopsy material
4 the frequency and types of anemia in an Ethiopian village (children as well as adults) with a high dietary iron intake as measured by simple tests, Hb PCV, ESR and in subsamples SI TIBC, serum folic acid and vitamin B 12 Previous studies (77) in the village chosen which is representative of the rural conditions on the Ethiopian highlands, had indicated a rather high frequency of anemia


ETHIOPIA
Field centres and stations belonging to the CHILDREN S NUTRITION UNIT
Field centre
Field station
Major town
Asmara
TIGRÉ
Gondar
SUDAN
Dessie
FRENCH SOMALIA
SOMALIA
Harar
IJAJI
ADDIS ABABA
ARUSSI
SIDAMO
KENYA
0 100 200 300 400 500
Kilometres

The country

Ethiopia is located in the Horn of Africa, immediately north of the Equator It is about $2^1/_2$ times the size of Sweden and has a population variously estimated at 20—24 milj

Almost two-thirds of the empire is a mountainous highland plateau, surrounded by regions of desert, savannah, lowland and expanses of volcanic rock Over 90 % of the people live in rural areas, primarily by a subsistence type of agriculture and animal husbandry (106) The population is composed of many tribes and several languages are spoken The official language is Amharic which is the mother tongue of about 1/3—1/4 of the population

The medical and health facilities include government and mission hospitals as well as mission clinics and governmental health centers and health stations In 1966 there were 70 hospitals (7 751 beds) 95 mission clinics, 63 health centers and about 480 health stations (16) The country as a whole had only about 370 physicians in 1966, all but 42 were foreigners employed on a more or less temporary basis All other health staff is in equally short supply and to a great extent concentrated to the capital and other urban areas

Due to incomplete vital statistics there is no reliable information as regards morbidity for the country as a whole However tuberculosis malaria leprosy, yellow fever helminthiasis, liver cirrhosis and, especially among children, malnutrition and intestinal infections are among the most commonly met diseases

The infant mortality rate has been studied in a few communities (106 111) where it has been found to range between 86 and 196 per 1 000 live births

Children s Nutrition Unit

The Children s Nutrition Unit (CNU) was started in the fall of 1962 with the following major aims according to the agreement between the Ethiopian and Swedish governments

To undertake a survey of the incidence of malnutrition in selected groups of Ethiopian children

To make a detailed analysis chemical and biological, of the food consumed in the families under study

To start an enrichment program with special emphasis on locally available indigenous foodstuffs

To make an evaluation of the physical fitness in relation to nutritional status in school age children and adults

To participate in nutrition teaching on various levels

The project was planned by a group of Swedish scientists who had been working on problems related to nutrition for many years Professor Bo Vahlquist (chairman), Professor O Mellander Professor G Ågren and Assistant Professor G Almgård who succeeded the late Associate Professor P E Nilsson The committee works in an advisory capacity to the Swedish International Development Authority (SIDA) and the project and members of the group usually spend a working period yearly in Addis Ababa

The base institution contains a well equipped laboratory for biochemical work and food analysis, an experimental kitchen, rooms for production of supplementary mixtures stores and offices for the different departments. The CNU is also in charge of a Children s Home nearby, where supplementary foods and weaning foods are tested

The total staff consists of about 10 foreigners and 70 Ethiopians

During the years 1963—1965 field surveys were performed in 5 different localities in the country selected in cooperation with a socio-anthropologist to be representative of the living conditions of large population groups. In two of these places (Addis Ababa and Ijaji, a village 220 km west of the capital) a long-term study of the growth, development and health condition of the children from 0—10 years was performed while in the other three cross-sectional studies were made (77). In addition to clinical examination, samples of blood, urine and stools were analysed and dietary surveys were made. It is estimated that the five test areas are representative of more than half the population of Ethiopia. The information and data thus obtained, especially those from the dietary surveys, have been used in an applied nutrition program with special emphasis on teaching nutrition on various levels. This program has been extended successively since 1965 as a weapon in the combat against the widespread malnutrition.

Weaning food recipes utilizing ingredients commonly used by the people have been worked out and tested and are now being spread through available channels including radio.

A supplementary feeding program started in late 1965. A number of mixtures had been worked out based on amino acid analysis of the (vegetable) protein ingredient and on biological testing (1). After acceptability tests on staff members and orphanage children a large scale supplementary feeding program was launched including several thousands of children. This proved quite successful and one of these supplementary mixtures was marketed under the name Faffa ("grow strong and healthy") in the spring of 1967.

SIDA has so far provided most of the funds for the project. The Ethiopian Government has provided counterparts to some of the foreign staff members. UNICEF has contributed machinery for the production of Faffa as well as dried skim which is included to 10 % in the mixture.

An important aspect of the work is to train Ethiopians to take over in due time. Scholarships have thus been provided for several of the senior Ethiopian staff members to study abroad and in-service training is going on systematically (117).

Dietary iron on the Ethiopian highlands

The source of the iron

Introduction

The main staple crop on the Ethiopian high land is tef, *Eragrostis abyssinica* As far as is known Ethiopia is the only country that uses tef as a cereal crop, though it is cultivated for its hay in other parts of Africa (76) The seed is sown at the end of the rainy season (June—September) and it is harvested in November—December traditionally by cutting the straws by hand with a sickle By that time the soil is very dry and dusty The threshing is done by making oxen walk around in the straws thereby expelling the grain This is done on a hard surface of soil and cow dung The grain is separated from the chaff by the time honoured method of throwing them up into the air on windy days The seeds are swept together and put into sacks

Before being milled the grain is cleaned A few handfuls are put on a strawplate and by small revolving movements of the plate combined with blowing most of the visible contamination is discarded

After milling (traditionally stone mills) the flour is mixed with water a piece of the previous dough is added and the mixture is left to ferment for 1—3 days after which a sourbread enjera is baked.

The individual tef grain is very small only the size of a pinhead The weight of 1 000 seeds is 0 3—0 4 g as compared with about 50 g for wheat (4) The relative surface is consequently very large and it has also minute husks which makes it difficult to clean completely

There has been some confusion however, as to the question of whether the iron is *in* or *on* the tef grain This may be of importance as regards the absorbability of iron The ICNND report (61) states that "The exceedingly high content of iron (approximately 100 mg of iron per 100 g of tef) may reflect some contamination of the cereal, but all samples analyzed as well as the prepared foods show this high iron content Analysis of tef washed with acid to remove adhering soil, etc , indicates that less than 10 per cent of the iron is due to contamination

Russel (quoted by Postmus (92)) found a mean value of iron of 90 mg/100 g and ICNND (61) 105 mg/100 g in tef as purchased

Almgård (4) cleaned the grain mechanically under a magnifying glass and found that the samples contained about 1 % soil which could be separated Analysis of tef cleaned in this way showed iron contents of 5 2 and 5 9 mg/100 g of dry matter for red and white tef respectively This would correspond to values found in wheat and barley

Melak Mengesha (76) had 12 white and 12 red tef strains grown in a field in Indiana USA and carefully avoided soil and iron contamination when harvesting He found that the iron content was 2—3 times higher in tef than in wheat, barley or grain sorghum (10 6—19 6 mg/100 g of dry matter for uncontaminated seed) and is not inclined to accept Almgard s low figures

Own studies

It seems important to differentiate between the iron in tef as purchased which usually has much soil contamination and the tef iron determined after various cleaning methods Some studies have been carried out at CNU to this end

The iron content in the reddish soil is high. In the CNU field center in Ijaji 9 soil samples from different tef fields were analysed for iron according to the same method as used for analysis of food iron (Schade *et al* (98) modified by K. Jacobsson (64)). The percentage of iron in these samples *ranged between 5.36 and 7.32*. Water from two different wells in the village contained 0.14 and 0.47 mg iron per liter, while the tap water in Addis Ababa (1 sample) contained 0.30 mg per liter, suggestive of a low solubility of the soil iron. The amount of iron in soils apparently varies from country to country but an average of 3.8 % has been reported (24).

Sergegna tef (red and white mixed) was purchased from 3 different sources (100 kg each). All had been harvested by hand. One was threshed by machine and the other two in the traditional way by cattle. The three samples were all cleaned at CNU by machine sifting and different kinds of contamination separated. The result is seen in Table 1.

Table 1. *Various kinds of contamination (% of weight) in 3 different tef qualities, bought at the local market, Addis Ababa*

	Straw, stones, excrements	Soil dust grit	Weeds, husks	Total
Sergegna tef Arsi Highest quality Machine threshed	8[illegible]	0 25	0 51	1 58
Sergegna tef Debre Zeit 1st quality Animal threshed	1 [illegible]4	0 15	0 4[illegible]	1 89
Sergegna tef unknown origin lowest quality and price Animal threshed	[illegible]	[illegible]	3	8 49

It is evident from these figures that the iron content of tef "as purchased" can be strongly influenced by contamination which in the lowest quality tef totalled 8.49 % of the weight.

An attempt was also made to determine the iron content in tef "as eaten" after different stages of mechanical cleaning and washing. Samples of white, red and mixed (sergegna) tef grown in the village Ijaji (CNU field center) were sifted and cleaned by hand in the traditional way (= tef "as eaten" *in the true sense*).

Subsamples of this were then washed with iron free water and hydrochloric acid (about 2 %) 20 times and again analysed for iron according to the previously mentioned method. The result is seen in Table 2.

Table 2. *Iron content in different types of tef after traditional cleaning and after further washing 20 times in dilute hydrochloric acid*

	mg Fe/100 g tef after traditional cleaning	mg Fe/100 g tef after further washing × 20 in dilute iron free HCl
White tef	57 9	13 0
White tef	79 4	3/ 9
White tef	35 8	14 5
Red tef	103 0	20 0
Red tef	97 0	24 8
Mixed (sergegna) tef	60 0	11 5
Mixed (sergegna) tef	30 1	27 4
Mixed (sergegna) tef	25 1	12 4

dry weight. Moisture in tef about 10 %

Generally the iron content was reduced to between 1/2 and 1/4 of the "as eaten" values after the washing procedure, indicating that most of the iron in tef "as eaten" is derived from soil contamination, which is contrary to what is stated in the *ICNND report (61)*. A comparison between results of different investigators is given in Table 3.

As can be seen the figures of the present author are in good agreement with those of Melak Mengesha. The red tef gave the highest iron values in both these studies, a little more than 20 mg per 100 g non-dried tef. This is approximately 4 times greater than the iron content in wheat or barley *(27)*. *It would* be tempting to conclude that the beautiful red color of this tef strain is derived from a high iron content, higher than in the white tef.

Table 3 *Iron content in "clean tef, according to 3 different investigators*

	mg Fe/100 g of dry matter in cleaned seed (Almgård 1963) (4)	mg Fe/100 g of dry matter in uncontaminated seed (Melak Mengesha 1964) (76)	mg Fe/100 g of dry matter acid washed seed (present study)
White tef mixed strain	5 9	11 5	13 0 14 5 36 9
Red tef mixed strain	5 2	19 6	20 0 24 8
Red white mixed (Sergegna)	5 5	15 5	11 5 12 4 27 3

Two values for iron in washed tef, one white tef and one mixed are in considerable disaccordance with others of the same tef type It is possible that some soil contamination may still adhere to the grain in spite of careful washing On the other hand there is also a possibility that there are genetically different strains with different iron contents This cannot be fully excluded in view of the varying results of different investigators

However the important thing is that tef which is by far the most common staple food on the highlands has a high iron content in the as eaten form

Determinations of soil iron were made from two more places in the Rift valley about 200 and 300 km south of Addis Ababa (at CNU field stations) In one (near Lake Zwai) the iron content was considerably lower than in Ijaji viz 1 51 % (1 sample) while in the other (near the provincial capital of Sidamo) it was comparable to that in Ijaji — 4 83 and 5 14 % (2 samples)

The important point, however is that tef is not grown in these areas (mainly corn sorghum and ensete the false banana) The high degree of contamination is firmly connected with tef, due to the special harvesting procedure and the size of the grain where in addition small husks facilitate the adherence of small particles of soil

Dietary iron intake

Ethiopia may represent the country with the highest intake in the world of iron per person per day, even greater than that of the Bantu natives in South Africa ICNND (61) found an average iron intake per person per day of 471 mg with a range of 98—1418 mg Dietary surveys (101) and food analyses (1) performed at CNU revealed the following as regards iron intake An adult man eats about 2—3 enjeras per day, each baked of 200—225 g tef Adult male shoe factory workers in Addis Ababa (5) had a mean iron intake of 520 mg daily with a range of 250—870 mg The consumption of iron in adult males was of the same order in the village Ijaji In government school children aged 9—11 and 12—14 years in Addis Ababa (5) a dietary survey (recall method) indicated an iron intake of 346 and 420 mg (range 121—730 mg) while for the same age groups in a private French school (Ethiopian children) it was a little lower 272 and 329 mg, respectively (range 5—680 mg) due to a smaller consumption of tef enjera In Ijaji in children from ½ year to about 5 years the iron intake ranged between 1 mg to about 50 mg, depending upon the amount of tef enjera consumed (101)

In general in the ordinary adult diet in tef areas the major part of the iron intake is

Hematological values in healthy and apparently healthy subjects of different ages in Addis Ababa, 2,400 m above sea level

Introduction

During the course of nutrition surveys performed by CNU over the years 1963—1965 (77) and in investigations made in the village Ijaji before the start of an applied nutrition program in 1965 (56) it became evident that a substantial part of the child population had marginally low hemoglobin values or frank anemia in spite of a high iron intake found in dietary surveys The need for a reference standard was often felt It appeared, however, that there were two main obstacles in obtaining such a normal child material The first was to get healthy non-infected subjects of different ages and who were in a satisfactory state of nutrition and the second was to over come the traditional fear of blood letting

As for adults it was considered that Ethiopian staff members of CNU and ESPC with easy access to medical attention if necessary and with a stable although for certain groups low income would be a satisfactory reference

An opportunity to obtain a hematological reference standard in children arose when examinations of presumably optimal children in the private French school were planned to get an anthropometric reference standard To this material of adults and school children, a series of pregnant women and newborn children were also added As it was not possible to obtain samples in the 1st class ward or 1st class antenatal clinic they were taken in the ordinary 3rd class clinic

The fear of blood letting is deep rooted and seems to depend upon the assumption that blood is not regenerated Even the small amounts of capillary blood used in finger prick tests caused a great stir in the village Ijaji and the whole base line examination in November 1965 was near to collapse when in addition a rumour was spread that the blood drawn was sold in Addis Ababa A common misbelief was also that the (Ethiopian) staff drank the blood from the people — apparently after they had been seen pipetting the samples The author has experienced several times that vital blood transfusions could not be given because the (compatible) father refused to give even 100 ml to his child with the statement that he himself would die get seriously ill or lose his potency On the other hand cautery bleeding cupping and the use of leaches is still quite widely practiced traditionally against various diseases (87) With increasing education this traditional fear is diminishing The Vacutainer technique facilitated matters as the blood obtained by venipuncture could be concealed in the tube by the examiner s hand or by a paper cone

Material

Adults The material consisted of 87 healthy and apparently healthy males and 50 apparently healthy non pregnant females most of them staff members of CNU and ESPC or in a few cases employed by foreign staff members of CNU 19 maintenance workers at the ammunition factory in Addis Ababa were also included

There were different kinds of professional and educational backgrounds There was the professional staff such as administrators nurses student nurses dressers laboratory technicians field assistants qualified nutritionists and nutrition workers — all with secondary school education or more and all comparatively well paid according to local conditions There were also unqualified or non professional" staff mem

and TIBC but also for Hb etc However the cord blood collected in heparinized Vacutainer tubes clotted in nearly all cases presumably partly because the tubes were not shaken properly and partly because the heparin may have whirled away when the stopper was removed from the vacuum tube Finger prick blood for determination of Hb and PCV therefore had to be taken from 30 other infants with a birth weight of more than 2 500 g within the first 36 hours of life (Nov —Dec 1967)

From 27 women venous blood was obtained during the last few hours before delivery except in one case when it was taken 3 hours after delivery

The collection of blood from the women in the antenatal clinic was made in consecutive cases one morning weekly at the delivery department this principle could not be upheld completely due to shortage of staff All newborn infants not older than 36 hours available 2 mornings a week were tested consecutively *

Methods

All blood samples collected were taken except in the newborns from the cubital vein with the subject sitting disposable needles were used From the newborns capillary blood for determination of Hb and PCV was taken after a finger prick and for SI and TIBC from cord blood which was allowed to drip freely from the placental part of the cord into iron free tubes Blood from children 1—14 years old and from adults was taken as follows after short or no stasis a disposable needle for Vacutainers was inserted in the vein and a 10 ml Vacutainer tube (iron free without additives) was filled with blood Within seconds about 1 ml of this blood was poured over to an Ellerman tube and pipetted for determination of Hb PCV and ESR Repeated checks showed that the whole procedure took only about 20 seconds after training and with 2 assistants taking care of the blood for pipetting During a period when no Vacutainers were available the blood from children in the French school and from adults was collected in two different tubes the first 1 ml in an Ellerman tube immediately taken care of for determination of Hb PCV and ESR and with the disposable needle still in place a second 10 ml iron free tube was filled for SI and TIBC determinations The time needed for this procedure was equally short

Blood from pregnant and delivering mothers was collected in two different Vacutainer tubes one with heparin with a 72 hour anticoagulant effect (for Hb and PCV) and one iron free without additives (for SI and TIBC) Immediately before pipetting the heparin tube was carefully and slowly rocked about 20—25 times to homogenize the blood again The maximal time between blood taking and processing for Hb and PCV was 2 hours

All blood for determination of SI and TIBC was centrifuged after clotting and the serum usually deep-frozen until processed after 1—2 weeks

All blood samples for determination of SI and TIBC were taken between 9 and 11 a m except in delivering mothers and from cord blood The subjects were not fasting as this was not possible from a practical point of view The morning meal is however very light and in most cases consists of tea with sugar and white bread without butter or cheese Only exceptionally was the colorimetric reading disturbed by turbidity

Hemoglobin (Hb) was determined as cyanmethemoglobin 25 µl of blood was mixed with 5 ml of ferri cyanide potassium cyanide solution (prepared fresh weekly from Metrix® dilutent tablets Michael Reese Research Found Chicago Ill USA according to instructions) and read in a Beckman C spectrophotometer at 540 mµ.

The instrument was calibrated at regular intervals The error of a single determination as calculated** from 30 consecutive duplicate determinations on capillary blood was 0 32 g % (the mean value of these reading was 11 73 g %)

Hematocrit (PCV) determinations were made according to the instruction manual for the M S E micro-hematocrit centrifuge Commercially available heparinized capillary tubes were used in duplicate The tubes were sealed at one end in a hot flame and centrifuged for 5 minutes According to the instruction manual the centrifuge should make 10 000 rev/min The reading was made in a hematocrit reader and the mean value of the readings of the 2 tubes was recorded It was seldom possible to seal and centrifuge the blood filled tubes until after ½—3 hours For various practical reasons the tubes could not be sealed with plastelina which gives a more sharp demarcation of the lower end of the packed cell column as compared to a more

---

* I am very grateful to Professor C Rendle-Short and to Drs R H J Hamlin and C Nicholson for permission to make these examinations and to the ward staff for their assistance

** The calculation was performed according to the formula

$$\sigma_i = \sqrt{\frac{\Sigma d}{2n}}$$ where d is the difference between duplicate determinations and n the number of differences

correspondingly, the mean corpuscular hemoglobin concentration remained unchanged The polycythemia was accompanied by a proportional elevation in the circulating reticulocytes and in the serum bilirubin, while the leucopoietic activity was not affected The polycythemia observed in persons just arriving at high altitudes seems to be due to a release of stored blood and hemoconcentration, while the polycythemia corresponding to repeated or constant exposure to a low barometric pressure is a consequence of erythropoietic hyperactivity and is adjusted to the degree of anoxemia

There was a striking inverse relationship *between the degree of arterial oxygen saturation* and the level of hemoglobin concentration in the circulating blood (see Fig 1)

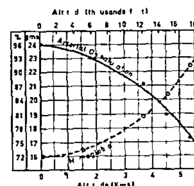


Fig 1 Hemoglobin concentration (g per 100 ml) and arterial oxygen saturation (%) in healthy male residents at different altitudes (Hurtado et al (57) courtesy Arch int Med)

Okin *et al* (86) studied normal hematological standards in adults at two medium high altitudes in the U S, 1 600 and 3 100 m When the mean values for hemoglobin in men were plotted in the curve (Fig 1) there was fairly close agreement Plotting of other reported mean hemoglobin values from different altitudes (47) shows a wider scatter around the curve, presumably due to differences in material and technique Walker (119) suggests an increase of hemoglobin concentration of 2 1 % for males and 1 8 % for females for every 1 000 feet elevation When plotted in the graph, hemoglobin values derived after using this correction factor will however, lie considerably above the curve This standard curve concerns male subjects

It is possible by interpolation in the curve to obtain a percent adjustment at a given altitude Evidence is lacking as to whether such a percent adjustment can be applied to *males females and children alike and whether* the same adjustment can also be applied not only to the mean hemoglobin values but also to a lower anemia limit The range of observations was wider in the higher altitude in the study of Okin *et al* (86), suggested to be due to increased individual variability of arterial oxygen saturation at higher altitudes If interpolation is made in the graph (Fig 1) for the altitudes of Ijaji (1,850 m) and Addis Ababa (2 400 m) and the mean value of 16 g % at sea level is accepted (see Fig 1) then approximately + 5 % for Ijaji and + 7 % for Addis Ababa should be used as correction factors This will correspond to roughly 1 g % for adults in Addis Ababa and a little less in Ijaji

It is obvious that more work is needed to get a definite answer to the question of the influence of medium high altitude on hemoglobin concentration For the purpose of this study however, it seemed reasonable to accept the per cent adjustments just mentioned, + 5 and + 7 % These then will be used when comparing mean values found with standard values and when discussing anemia rates

Results

1 *Adults*

The results of the determinations of Hb, PCV, ESR, SI and TIBC for adult males and females are recorded in *Table 4* Means and SD for MCHC and transferrin saturation are also entered In *Figs 2, 3 and 4* the percentage distributions of Hb, PCV and MCHC are shown

Although all the subjects were healthy or apparently healthy" 4 males (4 6 %) and 11 females (22 0 %) had an ESR above 20 mm (maximum 37 mm) 16 of the males (18 4 %) and 34 of the females (68 0 %)

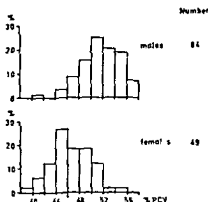


Fig 3 Relative distribution of PCV in healthy and apparently healthy males and females in Addis Ababa.

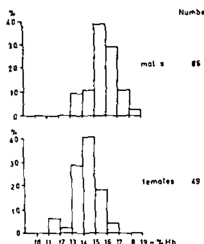


Fig 2 Relative distribution of Hb in healthy and apparently healthy males and females in Addis Ababa Interrupted vertical line indicates mean value

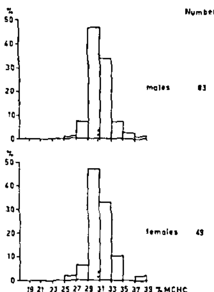


Fig 4 Relative distribution of MCHC in healthy and apparently healthy males and females in Addis Ababa

Table 4 *Hematological values in healthy and apparently healthy adult males and females in Addis Ababa*

		Age	Hb	PCV	MCHC	ESR	SI	TIBC	SI/TIBC
Adult males	M an	31 4	15 7	51 1	30 9	7 5	125	286	44 1
	S D		± 1 11	± 3 16	± 1 72	± 6 39	± 44	± 36	±16 6
		range							
	No	19—65	86	84	83	87	84	83	83
Adult females	Mean	23 9	14 2	46 0	30 9	16 0	94	308	31 1
	S D		± 1 05	± 3 42	± 2 00	± 7 92	± 41	± 52	±13 6
		range							
	No	16—45	49	49	49	50	50	50	50

non pregnant non lactating

Table 5 *Hematological values in ' professional and 'non-professional adults in Addis Ababa See text for explanation*

			Hb	PCV	MCHC
Males	professional	Mean	16 5	52 7	31.2
		S D	± 0 73	± 3 01	± 1 58
		No	25	25	25
	non professional	Mean	15 4	50 4	30 7
		S D	± 1 10	± 2 99	± 1 75
		No	61	59	58
Females	professional	Mean	14 4	45 8	31 4
		S D	± 0 88	± 2 99	± 1 74
		No	28	28	28
	non professional	Mean	14 0	46 4	30 2
		S D	± 1 22	± 3 98	±2 26
		No	21	21	21

had an ESR above 10 mm The mean Hb values for the subjects with ESR ≦10 mm were however, very close to those for the whole respective groups — in males 15 9± 1 05 g % (n=70) and in females 14 4±0 96 g % (n=16)

As the material was not homogeneous as regards educational and socioeconomic background an attempt was made to compare the hematological parameters in the professio nal and the non professional groups (cf Material) The result is shown in *Table 5* In both males and females, the professional group had higher mean values for Hb, PCV and MCHC (except for PCV in females) The differences for Hb and PCV between the two male groups were highly significant * The age range in the professional group was 20—35 years and in the non professio nal 19—65 *The mean Hb value in the latter* group for the age 20—35 (n=35) was the same as for all non professionals

No professional males and 4 professio nal females had an ESR above 20 mm The mean Hb value for professional males with ESR ≦10 mm (22 subjects) and that for professional females (13 subjects) were the same as for the whole respective professional groups

In Table 6, the mean Hb values for all adults and for professionals only are compared to altitude adjusted mean values given by Wintrobe (123) and Natvig *et al* (82) For comparison the mean Hb values reported by ICNND (61) for the country as a whole (the majority were residents at an altitude of about or more than 6 000 feet) are also given as well as those reported by Areskog *et al* (5) for shoe factory workers in Addis Ababa

Table 6 *Mean Hb values in adults in the present material compared to ' standard values (adjusted by + 7 % for altitude) and to two previous studies in Ethiopia*

	Males	Females
Present total material	15 7 (87)	14 2 (49)
Professionals only	16 5 (25)	14 4 (28)
Foreign staff CNU	16 7 (11)	—
Wintrobe (123)	17 1	15 0
Natvig *et al* ** (82)	16 8	15 3
ICNND country mean (61)	14 4	13 2
Areskog *et al* (5)	15 6 (42)	—

number of subjects
adjusted by + 7 % for altitude

* Throughout this study the following definitions will be used Almost significant P <0 05 significant P <0 01 highly significant P <0 001

The mean Hb value for 11 male foreign healthy staff members in Ethio-Swedish projects, who had been resident in Addis Ababa for more than ½ year, is also recorded

As will be seen the mean Hb value for the total Ethiopian males was almost identical with that of shoe factory workers examined by Areskog *et al* but was more than 1 g % higher than ICNND s mean value for the whole country It was however more than 1 g % lower than the altitude adjusted values given by Wintrobe and Natvig The mean value in the male 'professional staff and that in foreign staff members agreed well however, with these standard values

The total females, although having a higher mean value than the ICNND value for the whole country, were slightly below altitude adjusted standard values This also held for professional females alone, the difference from Natvig s mean value being 0 9 g % and from that of Wintrobe 0 6 g %

The mean values for SI and TIBC in subjects with ESR ≦10 mm were very close to those for the whole respective groups, in males with a low ESR, the SI value was 129 µg % compared to 125 µg in the whole group and in females 90 µg % compared to 94 µg % in the whole group

When only professionals' with ESR ≦10 mm were included, the mean value for SI still remained at the same level males 125 µg % and females 92 µg %

The same was true for mean TIBC values in subjects with a low ESR, although in male professionals with a low ESR the mean value was slightly raised from 266 to 304 µg % This difference is not significant

Anemia rate

Any discussion about anemia rates will be hampered by the recognized uncertainty of how to define anemia and of the hemoglobin level below which anemia can be said to exist This problem is even more difficult when altitude adjustment comes into the picture In clinical work the use of the term marginal zone values is to be preferred to a sharp anemia limit ICNND, in surveys in Bolivia (60), has differentiated the Hb values as high, acceptable, low and deficient for different altitudes For the altitude 2,400 m high would be above 16 0 g %, 'acceptable 14 5—16 0 g % low 12 5—14 5 g % and deficient below approximately 12 5 g %

For the sake of discussion in this paper it is assumed that the adjustment of +7 % for the Addis Ababa altitude is also applicable to suggested lower hemoglobin limits below which anemia can be said to exist' although it is recognized that such an assumption may be debatable

In *Table 7* the percentage of anemic adult subjects is given, using 3 different altitude adjusted standards

Table 7 *Percentage of anemia in adults in Addis Ababa according to 3 different criteria with adjustment for altitude*

	WHO (62)	Wintrobe (123)	Natvig *et al* *** (82)
Males	7 0	19 8	19 8
Females	8 2	8 2	16 3

males < 13 9 g %
females < 12 8 g %
(see text)

males < 15 0 g %
females < 12 8 g %

males < 15 0 g %
females < 13 4 g %

It is obvious that with different comparison standards the percentages of anemia in the present material will vary a great deal This would be the case even if the standards were not altitude adjusted The large differences in anemia rates when different standards are applied also indicate that a number of cases are marginally low

The range of Hb values in the present material for males was 13 2—18 6 g % and for females 11 6—16 3 g % 6 males and 4 females had Hb values below the anemia limit recommended by WHO (62), adjusted

for altitude viz 13 9 g % for males and 12 8 g % for females *

Two of the 6 males had a moderately raised ESR (24 and 29 mm) In 1 case there was no record of PCV but the remaining 5 had a PCV below the mean value for the whole group although all were within the mean — 2 SD In all these 5 MCHC was below the mean value for the whole group but in only 1 of them was it below the mean — 2 SD In the latter subject SI was 119 and TIBC 247 µg % Finally in all 6 SI was below the mean value for the group It should be pointed out that all the 6 anemic males had stainable hemosiderin in the bone marrow and that all belonged to the non-professional group It should also be noted that 4 of the 6 males were above 45 years of age In the total material only 14 % exceeded 45 years

Two of the 4 females had a moderately raised ESR (33 and 35 mm) In all the 4 the PCV and MCHC values were below the mean for the whole group One had a PCV value below the mean — 2 SD for the whole group and another had MCHC below the mean — 2 SD The last mentioned had delivered 8 months earlier Her SI value was however 80 µg % combined with a low TIBC and therefore did not present a picture typical for iron deficiency anemia Two had SI values below and 2 above the mean for the whole group One anemic female was professional

In 5 females and no males SI was below 50 µg % slightly lower than the normal limit given by Wintrobe (123) None were anemic according to previously discussed criteria but all had an ESR above 10 mm although only 2 had definitely pathological values (34 and 37 mm) suggestive of recent or present infection

---

* WHO recommended in 1959 (62) a lower limit of 14 0 g % for males 12 0 g % for non pregnant females and 10 0 g % for pregnant females These limits have been revised in 1968 and the new recommended anemia limits will be 13 0, 12 0 and 11 0 g % respectively (Professor L Hallberg personal communication) They will be used throughout this paper

Comments

Areskog *et al* (5) on examining Air Force cadets in Debre Zeit, outside Addis Ababa, at an altitude of about 1,900 m, found in this homogeneous group of physically fit young men a mean Hb of 15 9±0 9 g % A minor upward correction of about 0 2—0 3 g % as according to the above discussion on this topic, would make a comparison to the Addis Ababa altitude possible In the same study (5) the mean Hb in shoe factory workers in Addis Ababa was found to be 15 6±1 2 g %

The present series, although heterogeneous from an educational and socio-economic point of view, was considered medically fit, and, although no special dietary data were obtained all subjects were known to consume the traditional Ethiopian diet, meaning a high dietary iron intake The intake of other nutrients necessary for hematopoiesis was not specifically studied although the protein intake can be assumed to correspond to or even especially in the professional groups, exceed that of the shoe factory workers in the study of Areskog *et al* (5), at least as regards the intake of animal protein

The mean Hb value of 15 7 g % in males in the present material agrees well with those in the two materials of Areskog *et al* although there is a difference of slightly more than 1 g % from altitude adjusted standard values In the selected group of professionals however the mean Hb value of 16 5 g % came close to the reference standard used and would be in agreement with other standards for instance that proposed by Vahlquist (116) (16 5 g % when adjusted by +7 % for altitude)

The difference between the mean Hb in males and females in most standard materials is usually about 1 5—2 g % This is true also for the present material where the difference is 1 5 g % increasing to 2 1 g % if professional males and females are compared The difference between the mean Hb for all females in the present material and the altitude adjusted mean values of the reference

standards used (Table 6) is 0 8—1 1 g %, but only 0 5 g % when comparison is made with the altitude adjusted mean value of 14 7 g % proposed by Vahlquist (116)

In view of the fact that agreement between different standard values published is by no means complete due to differences in material and techniques, and the rather imprecise way of choosing the factor for altitude adjustment, the mean values obtained in the selected groups of young and medically fit professionals in the present material can be considered reasonably comparable to other standard values reported

The cases of anemia in males diagnosed according to the criteria of WHO were apparently not due to iron deficiency, as all had stainable hemosiderin in the bone marrow, most of them in abundance The fact that 4 of the 6 males diagnosed as anemic belonged to the oldest in the material (49 years or more) cannot be satisfactorily explained There was a successive decrease in mean Hb from 16 0 g % in subjects below 25 years to 15 1 g % in ages ≧45 although this may be explained by the fact that the younger groups included the professional staff ICNND (61), however, reports the same decline in mean Hb with increasing age

The state of the iron stores in adult healthy Ethiopian females is not known although it is reported from South Africa (10) that Bantu females with a high iron intake had much less siderosis than males and that about 75 % had iron concentrations in the liver which were normal or very close to normal The 4 females in this material did not present any picture typical of iron deficiency All females except 1 were below 45 years, so that an age group comparison with the males was not possible

The mean value of 125 μg % for SI in males is in satisfactory agreement with that for other workers while the value for females, 94 μg %, is slightly lower than most reported mean values (8) In both males and females the mean values for TIBC were slightly lower than those mostly reported (8)

There are no indications that chronic exposure to high altitudes significantly affects SI or TIBC (95, 112)

2 "Privileged children 1—14 years

The results of determination of Hb, PCV, ESR, SI and TIBC as well as the results of MCHC and transferrin saturation for the different age groups are given in Table 8 The sexes are not treated separately Figs 5, 6 and 7 show the percentage distributions of Hb, PCV and MCHC

Although, as mentioned previously, all the children were in good health and none showed any signs of relevant skin infection or other infectious ailments at the clinical examination, a considerable number had a raised ESR In the age group 1—4 years 28 1 % had an ESR

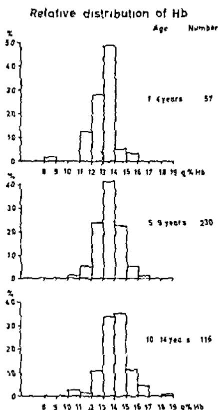


Fig 5 Relative distribution of Hb in privileged children 1—14 years in Addis Ababa

higher than 20 mm (maximum 39 mm) in the group 5—9 years 15 4 % (maximum 40 mm) and in the group 10—14 years 12 3 % (maximum 36 mm) The falling rate is in agreement with the decreasing mean ESR with increasing age (cf Table 8) The mean Hb value for the children with ESR ≤20 mm was however very close to that for the whole respective group — in the age groups 1—4 and 10—14 years it was only 0 1 g %

Table 8 *Hematological values in 'privileged children' 1—14 years* in Addis Ababa*

		Hb	PCV	MCHC	ESR	SI	TIBC	SI/TIBC
1— 4 years	Mean	12 9	42 0	30 8	17 5	89	323	28 9
	S D	± 1 09	± 2 43	± 1 97	± 7 47	±30	± 50	± 9 5
	No	57	51	51	57	26	26	26
5— 9 years	Mean	13 5	43 8	30 8	13 3			
	S D	± 0 97	± 2 98	± 2 09	± 7 45			
	No	230	222	222	227			
						93	305	30 5
						±35	± 44	±10 0
						82	82	82
10—14 years	Mean	13 9	44 8	31 1	12 7			
	S D	± 1 18	± 2 54	± 2 20	± 6 95			
	No	115	114	114	114			

* both sexes

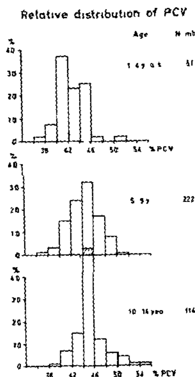


Fig 6 Relative distribution of PCV in privileged *children 1—14 years in Addis Ababa*

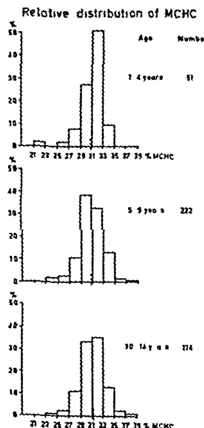


Fig 7 Relative distribution of MCHC in *"privileged children" 1—14 years in Addis Ababa*

higher while it was exactly the same in the 5—9 year age group When, however, only children with ESR ≦10 mm were included, the mean Hb values for the three age groups increased slightly and were almost exactly the same as the altitude adjusted values given by Wintrobe (123) and Natvig *et al* (83) (see *Table 9)* It appears that in the age group 1—4 years 78 9 % had an ESR above 10 mm in the group 5—9 years this value was 59 6 % and in the group 10—14 years 54 8 %

The mean values for PCV in children with ESR ≦10 mm were also slightly higher, making the mean MCHC value remain at the same level

The means for SI and TIBC in children with ESR ≦10 mm in the 5—14 year age group were 98 and 306 μg %, respectively, (n=37), thus close to the mean values for the whole group In the group 1—4 years, SI and TIBC were recorded in only 5 children with ESR ≦10 mm

Anemia rate

As with the adults the same altitude adjustment (+7%) will be applied to some suggested lower hemoglobin levels below which anemia can be said to exist In *Table 10* the anemia limits suggested by WHO (62) and Natvig *et al* (83) are used as references WHO proposes for the age group ½—4 years 10 8 g % for 5—9 years 11 5 g % and for 10—14 years 12 5 g % Natvig *et al* gives for children 7—9 years a lower limit of 11 1 g % and for 10—13 years 11 6 g %

Table 10 *Percentage of anemia in "privileged children' 1—14 years according to criteria (altitude adjusted) of WHO* (62) and Natvig et al** (83)*

	WHO (62)	Natvig *et al* (83)
1— 4 years	5 3	—
5— 9 years	7 8	5 7
10—14 years	30 4	7 0

* ½ — 4 years Hb <11 6 g %
5— 9 years Hb <12 3 g %
10—14 years Hb <13 4 g %

** 7— 9 years Hb <11 9 g %
10—13 years Hb <12 4 g %

In view of the fact that Natvig s proposed normal values are derived from a very carefully checked and iron supplemented series it would seem probable that the anemia limit suggested by WHO for children 10—14 years is too high This is also supported by the normal ranges given by Vahlquist (116) and Wintrobe (123)

Table 9 *Hematological values in children with ESR ≦10 mm, compared to altitude adjusted 'standard values' for Hb*

		Hb	PCV	MCHC	Wintrobe (123)	Natvig *et al** (83)
1— 4 years	Mean	13 4	43 5	30 8	13 4	—
	S D	± 0 83	± 3 06	± 1 33		
	No	12	10	10		
5— 9 years	Mean	13 6	44 4	30 6	13 8	13 6
	S D	± 1 16	± 2 60	± 2 47		
	No	93	89	89		
10—14 years	Mean	14 3	45 2	31 6	14 3	14 1
	S D	± 1 17	± 2 66	± 2 01		
	No	52	52	52		

adjusted for altitude by + 7 %
7— 9 years
10—13 years

In an analysis of the children who were anemic according to the criteria of WHO, the following was found

1—4 years 3 out of 57 were anemic according to WHO (Hb range 8 1—11 3 g %) One child, 1 year old had a marked hypochromic anemia (Hb 8 1 g % and MCHC 22 5 %) The other 2 had marginally low Hb values of 11 1—11 3 g % MCHC recorded in 1 of those was 27 8 % The ESR values ranged between 18—28 mm

5—9 years 18 out of 230 were anemic according to WHO (Hb range 10 3—12 2 g %) In 8 of them MCHC was below 27 % (9 in the whole age group) In 3 of the 18 the ESR values were above 20 mm or about the same percentage as for the whole age group

10—14 years 35 out of 115 were anemic according to WHO (Hb range 10 4—13 3 g %) In 4 of these 35 MCHC was below 27 % In 3 of them the ESR value was above 20 mm — or about the same percentage as in the whole age group 5 children (4 3 %) had a Hb value below 12 g % and 17 (14 8 %) below 13 g % indicating that the majority of the anemic cases were marginally low Only 8 children were anemic according to the altitude adjusted criteria of Natvig In 4 of these MCHC was below 27 %

In 8 of 108 children the SI value was below 50 µg % (range 30—49) None of these 8 had an ESR above 20 mm or were anemic according to any criteria discussed

Comments

The mean Hb values especially in children with ESR ≦10 mm were in good agreement with the altitude adjusted standard values given by Wintrobe (123) and Natvig *et al* (83) The values for the three age groups were also in agreement with altitude adjusted values proposed by Vahlquist (116) for the ages 3 7 and 11 years, respectively

Further, they agreed with the means given by Areskog *et al* (5) for children tested for physical fitness at the French school in Addis Ababa ICNND (61), using a different age group system found for school age boys ≦9 years a mean Hb of 12 5 g % and for school age boys below 15 years 13 7 g % (the girls had nearly the same mean values), which seemingly is a little lower than in the present study The mean MCHC for these groups were the same as in this study

The mean values for SI in ages 1—14 years (Table 8) were slightly lower than means reported by Hagberg (40) but agree better with those of Vahlquist (115) The mean values for TIBC were also lower than those reported by Hagberg (40)

The total anemia rate is difficult to evaluate in view of the considerable difference in the age group 10—14 years between the two criteria of WHO (62) and Natvig *et al* (83) As already indicated it would seem that the anemia limit suggested by WHO in this age group is a little too high If that of Natvig is applied for this age group and those of WHO for ages 1—9 years the total anemia rate would be 7 2 %

MCHC has been *claimed to be an insensitive* parameter for diagnosing iron deficiency anemia (32) In adults and in children the mean MCHC in this study was about 31 % with 2 SD about 4, which also corresponds to the variation given by Natvig *et al* (83) in subjects 7—20 years old Throughout this study an MCHC value of below 27 % will be used to denote hypochromia in anemic subjects suggestive of iron deficiency anemia, although the anemia of chronic infection may sometimes be hypochromic (123) In view of findings of Garby *et al* (32) it is possible that the rate of iron deficiency anemia may be underestimated especially in situations where *iron deficiency* is not uncommon

According to this criteria and the anemia criteria of WHO (62) for the age groups 1—9 years and those of Natvig *et al* (83) for 10—14 years 3 4 % of the children had an iron deficiency anemia

Dietary data (101) indicate an increasing iron intake once the children start to participate in the adult iron rich diet In the French school in the age group 9—14 years a daily average iron intake of about 300 mg was found (5)

No stool tests were made in these children in the present study and although hookworm is not seen in Addis Ababa a few children with suggestive iron deficiency anemia may have brought an infestation from the rural areas The one year old child referred to previously, most certainly had developed anemia due to an iron deficient monotonous milk diet

The high mean values for ESR and the high rate of children with an ESR of more than 20 mm should be noted in view of the fact that the clinical examination did not reveal any signs of infections However, in the surveys of ICNND (61) and CNU (77) high levels of gamma globulins have been found already from early ages ICNND (61) reported that the gamma globulin was elevated in all but the breastfed infants These findings are suggestive of early contraction of significant but often subclinical infections The levels of the immunoglobulins G and D have been found highly significantly raised above those found in Swedish children of the same age (66)

3 Pregnant women and mothers at delivery

The results of the determinations of Hb, PCV, SI and TIBC as well as MCHC and transferrin saturation are given in Table 11 The hematological parameters of the mothers at delivery are also recorded and for comparison those of non pregnant, apparently healthy females

The range of Hb in the expectant women was 10 7—15 8 g % and in the mothers at delivery 11 1—16 3 g % A total of 5 women or 6 6 % would be classified as anemic if the new WHO recommendations (see footnote page 25 were followed — a lower limit of

Table 11 *Hematological values in pregnant women and mothers at delivery (compared to non pregnant females) in Addis Ababa*

		Age	Hb	PCV	MCHC	SI	TIBC	% satur
7th month	Mean	26 4	12 6	40 9	31 0	91	370	25 4
	S D		± 1 06	± 4 34	± 2 88	± 34	± 50	±11 1
	No	18—36	9	9	9	9	9	9
8th month	Mean	24 3	13 1	42 3	30 7	104	417	25 6
	S D		± 1 11	± 3 91	± 2 24	± 24	± 60	± 7 6
	No	17—39	22	22	22	21	21	21
9th month	Mean	25 6	13 4	44 2	30 4	137	402	34 3
	S D		± 0 88	± 3 41	± 2 53	± 53	± 40	±13 7
	No	16—37	21	21	21	20	20	20
7—9th month	Mean	25 2	13 1	42 8	30 6	115	402	29 1
	S D		± 1 03	± 3 92	± 2 43	± 43	± 53	±11 6
	No	16—39	52	52	52	50	50	50
At delivery	Mean	26 0	13 8	45 1	30 6	82	380	21 3
	S D		± 1 20	± 2 84	± 2 02	± 42	± 62	±10 0
	No	16—43	24	24	24	26	26	26
Non pregnant females 50	Mean	23 9	14 2	46 0	30 9	94	308	31 1
	S D		± 1 05	± 3 42	± 2 00	± 41	± 52	±13 6

age range
number See Table 4

11 0 g % adjusted by +7 % for altitude = 11 8 g % One was delivering and 4 were in the 7—8th month of gestation Their Hb values were marginally low, 10 7—11 7 g %, and all had MCHC values above 27 % Their SI ranged between 61 and 106 µg %

As can be seen in Table 11 there was a gradual increase in the mean Hb and PCV values from the 7th month of gestation up to the time of delivery, while the mean MCHC remained at about the same level At delivery the mean Hb PCV and MCHC values were close to those in non-pregnant females The differences in Hb and PCV between the 7th and 9th months were almost significant The mean SI value also showed a gradual increase up to the 9th month, when in fact it exceeded that of non pregnant females (highly significant difference) The mean value at delivery is not fully comparable as blood from these women was drawn at any time of the day, while in the expectant mothers it was taken only at 9—11 a m The mean TIBC values remained throughout, with moderate fluctuations, on a markedly higher level than those in non pregnant females The % transferrin saturation at the 9th month slightly exceeded that in non pregnant females When the expectant women (7—9th month) were divided according to *parity* (1st pregnancy (n=10) 2nd—3rd pregnancy (n=15) and ≧4th pregnancy (n=27)) the mean Hb values showed no consistent trend — for the three groups they were 13 3, 12 6 and 13 4 g % respectively The mean values for SI, on the other hand showed an upward trend — 92, 103 and 129 µg % for the three groups respectively The difference between the lowest and the highest values is almost significant The mean ages in these three groups were 21 9, 21 8 and 28 3 years, respectively

When the expectant women were divided according to *age* groups of 15—20 years (n=9), 20—29 years (n=30) and 30 years or more (n=13) the mean Hb values still showed no consistent trend — 13 4 13 0 and 13 2 g % respectively The mean values for SI, on the other hand, showed an upward trend — for the 3 groups these were 96, 117 and 124 µg % respectively The difference between the lowest and highest values is not significant

The same was found when the women were divided into only two age groups 15—24 years (n=25) and ≧25 years (n=27) Here the mean Hb values were 13 2 and 13 1 g % and the mean SI values 101 and 127 µg %, respectively The difference is almost significant

Comments

In normal pregnancy the total blood volume increases by about 35 % Most of this is due to an increment in the plasma volume with only a modest increase in the red cell volume The plasma volume increases up to about the 34th week after which there is a decline while there seems to be a linear increase in the red cell volume up to term (59)

In the peripheral blood the hemoglobin concentration usually falls, starting already during the first trimester, parallelled by a fall in the hematocrit and red cell count (59) The minimum is reached at about the 30th —32nd weeks or a little later, followed by a slight rise at the end of pregnancy (59) MCHC appears to vary little during pregnancy, although in some series (59, 109) a slight fall has been reported Sturgeon (109) found a fall from 31 6 % in early pregnancy to 30 9 % in late pregnancy in women who received no iron, while iron treated groups showed no such reduction Many workers have reported (see Hytten *et al* (59)) that the mean hemoglobin concentration in women given therapeutic doses of iron by mouth or parenterally rose during pregnancy, sometimes to non-pregnant levels and that the usual fall in hematocrit could be similarly modified by iron Gerritsen and Walker (33) have reported that in Bantu women with an exceptionally high habitual dietary intake no

fall in mean hemoglobin concentration or mean hematocrit occurred during pregnancy

The average serum iron concentration is usually reduced to about 35 % below the mean for non-pregnant women and the transferrin level raised, resulting in a low percentage saturation (59), making the picture resemble that in iron deficiency anemia Iron medication can arrest or modify this fall in serum iron (109) In the Bantu women there was no reduction at all, although the transferrin levels increased (33)

In the present material there was a difference of 1 6 g % between the mean Hb value in the 7th month of gestation and that of *non pregnant females* *What is interesting is* that it successively increased up to parturition coming very close to the non pregnant mean value and being parallelled by a corresponding rise in the mean PCV value The mean Hb value for all pregnant females including those at delivery was 13 3 g % This is less than 1 g % lower than that for non pregnant females and although this difference is greater than that reported from South Africa in Bantu females (33), it is smaller than in most reported series without iron treatment (59) ICNND (61) found in Ethiopian pregnant women at the same altitude as the present investigation, a mean Hb of 14 0 g % in the first trimester and of 13 1 g % in the third or the same as in this study and also the same mean PCV and MCHC Only 2 of the 96 examined pregnant females in their series showed evidence suggestive of iron deficiency anemia

Also the mean value for SI increased from the 7th month and onwards and almost significantly exceeded that of non pregnant females in the 9th month The median value in this group, 116 µg %, is, however, probably more representative than the mean value of 137 µg %, which may be distorted by two very high values, well above 200 µg % The same also applies to the high mean values found in those pregnant for the 4th or more times (mean value 129 µg %, median value 116 µg %) and those ≥25 years (mean value 127 µg %, median value 114 µg %) The almost significant difference between these means and those in the lowest parity group and age group, respectively, is therefore questionable The mean value for SI for all expectant women 115 µg % (median 111 µg %) is in good agreement with mean values reported for non-pregnant females (40, 115) and is slightly, although not significantly, higher than the mean value in non-pregnant Ethiopian females In any case, the usually recorded decrease in SI of about 35 % compared to non pregnant values was not met here and the findings are in agreement with the value reported for pregnant Bantu females in the last trimester, 129 µg % (33)

The increase in the TIBC levels in the present material also corresponds to that in pregnant Bantu women, where the mean value in the last trimester was 403 µg %, compared with 323 µg % in non-pregnant females (33) In the present material the corresponding values were 402 and 308 µg %, respectively

The similarity to the findings in pregnant Bantu women who also have a high dietary iron intake is pronounced

There is evidence that iron absorption during pregnancy is increased severalfold Hahn *et al* (42) found that from the 15th week of pregnancy the iron absorption started to increase and after week 34 it was nearly 4 times that of weeks 10—14 Hallberg and Rybo (97) found an increased absorption of between 2 and 17 times Their subjects had first received iron injections to exclude the possibility that an iron deficiency would promote improved absorption They concluded that it is not the availability of iron in the body *per se* which is responsible for the increased iron absorption but rather that the iron needs of the fetus, placenta and bone marrow are so high that the mobilization from the stores cannot keep pace

It seems conceivable, therefore that in the present material the pregnant females not

given iron medication but having a habitually high dietary intake of iron would absorb more than those in the non-pregnant state and that this additional iron absorbed — as in iron medication — would in the first place prevent iron deficiency anemia and secondly would result in only a slight or moderate fall of the hemoglobin concentration, also arresting the usual fall in serum iron concentration — all laboratory signs which have been ascribed to iron deficiency (41, 109) The possibility that increased mobilization from presumed large iron stores occurs during pregnancy must also be kept in mind although in non pregnant Bantu females the stores were essentially within the normal range (10)

4 *Newborn infants*

The results of the determinations of Hb, PCV, SI and TIBC as well as MCHC and transferrin saturation are given in Table 12 It should be noted (cf Material') that determinations of Hb and PCV were made on capillary blood and SI and TIBC on cord blood (on other infants)

As is evident from the high figures of standard variations from the means of Hb and PCV the individual values were widely scattered, although the mean MCHC agreed well with that of all other age groups The range of Hb values was 12 3—28 9 g % and that for PCV 41—85 % The two extreme Hb and PCV values were obtained from the same two children One forceps delivered girl with a birth weight of 2,700 g was most certainly anemic (Hb 12 3 g %, PCV 41 % and MCHC 30 0 %)

The high SI and low TIBC should be compared to the corresponding values in mothers at delivery who in this material, as in others previously referred to, had considerably lower SI and higher TIBC values

Comments

It is difficult to compare the mean values for Hb and PCV with 'standard values' as the relative intra-uterine hypoxia in the mothers, who are already affected by the relative hypoxia at the altitude of 2 400 m may justify a higher correction factor than +7 % (cf Fig 1) In addition normal values reported for capillary blood at birth (1st day) vary a great deal It has also been shown (85) that in infants with the cord clamped late there is a temporary rise in the capillary PCV value with a maximum at 6 hours after birth followed by a fall in the next 6 hours back to approximately initial values, which persist for the next 2—3 days In the study of Oh and Lind (85) it was also shown that the capillary hematocrit values were higher when taken from pin-pricks in warmed than in unwarmed heels The majority of the infants in this material were tested between 6 and 24 hours after birth and always by pin pricks in unwarmed finger tips In the obstetric routine at the hospital where the tests were made the cord is not clamped until all pulsations have ceased

Vahlquist (115) found in capillary blood on the first day a mean Hb value of 18 4± 2 62 g % Sturgeon (110) gives 18 1±2 3 and

Table 12 *Hematological values in newborn infants in Addis Ababa*

	Hb	PCV	MCHC	SI	TIBC*	% satur
Mean	21 0	68 3	30 9	150	267	57 3
S D	± 3 14	±10 37	± 2 90	± 55	± 59	±19 3
No	30	25	25	47	44	44

Capillary blood at <36 hours age
Cord blood

Moe (79) a value of 19.8 ± 2.4 g % (2nd—6th day). Albritton (2) reported higher values and gave for the 1st day a value of 21.2 g % with a range of 17.7—26.5 g %.

The mean Hb value given by Wintrobe (123), which was compiled from several sources, adjusted by +7 % for altitude, will be 20.9 g %, which compares favourably with the mean value in the present material, although as was pointed out, the altitude correction factor should possibly be higher. The PCV value is even more difficult to compare with standard values due to probable differences in technique.

The mean value for SI agrees with the findings of Vahlquist (115) and Laurell (67) but is lower than that reported by, for instance, Hagberg (40) and Sturgeon (110). The marked difference in TIBC between the maternal and the cord blood is in good agreement with the findings of others (40, 67, 110) as well as the great difference in transferrin saturation.

Sturgeon (109, 110), in a carefully controlled material of pregnant mothers, one group of whom was given no iron and two other groups iron supplemented, found no difference in mean Hb and SI in the offspring in the three groups at birth and up to 18 months. With respect to the high dietary iron intake in the mothers, the hematological picture in the newborn as shown in this material displays no particular signs which could be attributed to excessive maternal iron load.

Summary

A total of 615 healthy and apparently healthy subjects aged 1—65 years were examined hematologically. In addition the capillary blood was examined in 30 newborn infants and the cord blood in another 47.

In general the mean hemoglobin values in the different age and sex groups agreed well with accepted "standard values", adjusted for altitude by +7 %. In adult males there was a significant difference in mean Hb between professionals and non-professionals, only the former group being comparable to "standard values" and to the mean value found in foreign residents. In adult females the corresponding difference was smaller and non-significant.

In children aged 1—14 years there was good agreement with "standard values", *especially in children with a low ESR. There* was also a satisfactory agreement in newborn children.

In pregnant and delivering mothers there was only a comparatively small decrease in mean Hb values compared to non-pregnant females.

The mean MCHC was approximately 31 % in all groups.

The mean serum iron level remained constant from before 5 years and upwards, with an increase to higher values after puberty in males, which is a pattern observed in other investigations, while TIBC rather exhibited the opposite pattern with higher values in early childhood and lower values especially in adult males, also confirming the findings in other studies.

Contrary to what is usually observed, the serum iron in the pregnant females in the 3rd trimester was not decreased compared to non-pregnant females.

The anemia rate, according to the criteria discussed, was low, approximately 7—8 %. No definite cases of iron deficiency anemia were diagnosed in adults — identified as anemia with MCHC below 27 % — while in children 1—14 years about 3 % appeared to have this type of anemia.

It would therefore be justified to conclude that the iron nutrition on the whole seemed to be adequate in the subjects under study.

Evaluation of the iron stores

Introduction. Oral iron overload

The body of the normal adult male contains about 3—4 g iron (8). Approximately 65 % of this iron is contained in the circulating hemoglobin, some 30 % is storage iron and the remainder is to be found in myoglobin, the heme enzymes and in the plasma (8).

After adulthood is reached, about 1 g storage iron is present in the body (8). This store remains essentially unchanged in males during the remainder of life since the amounts of iron absorbed are normally sufficient to balance the small amounts excreted. In women, menstruation and pregnancy may cause a drain and even depletion of the stores. An absolute increase in the iron content of the body means that more iron has been absorbed than excreted or that iron has been introduced via some other route. In both instances the excess iron is laid down in stores in the bone marrow, liver and spleen as ferritin and hemosiderin (8).

The average healthy adult male in temperate climates loses normally about 0.6 mg of iron daily in the stools, urine, sweat, hair and desquamated cells (8). A female loses approximately double that amount during the reproductive period (8), however, with considerable individual variations (84).

The absorption, in contrast to the excretion, may vary within a wide range. Iron in the divalent, ferrous form is more easily absorbed than in the trivalent ferric form (14). The absorption is favoured by large doses of ascorbic acid (15), mainly due to its reducing action in the intestines, while phytates, phosphates and excess calcium may have an unfavourable effect on the absorption of iron although their importance in the normal diet is uncertain (81). The role of the gastric juices in iron absorption is subject to conflicting views but it appears that pancreatic secretions may have an inhibitory effect (8).

The presence of clay in the food (geophagia) has been shown to diminish absorption (78). There are good reasons to believe that general bulkiness of the food, as is common in developing countries, will have an unfavourable effect on iron absorption. However, surprisingly few facts are available in this field.

The amount of iron in the tissue stores is also of importance. A decrease results in enhanced absorption and *vice versa* (11, 91). There is increased absorption when erythropoiesis is stimulated by bleeding, by hemolytic agents, by cobalt (11) and by the relative anoxia of high altitudes (95), while the opposite occurs when erythropoiesis is depressed (11, 95).

A gross increase in the body iron can arise from a habitually high dietary iron intake as among the Bantu (7, 21, 22, 38, 53, 118) or from habitual iron medication over several decades (114). In idiopathic hemochromatosis the excessive iron deposits have been ascribed to an increased absorption from a normal diet due to a presumed metabolic error (8), although this view has been questioned (71, 72). It is also seen in certain anemias and as a result of frequent transfusions (for comprehensive reviews see refs. 8 and 71). An excessive dietary iron intake has been reported to occur primarily among the Bantu population in Southern Africa (120) and in Ethiopia (61). From some other parts of the world, a high iron intake has been connected with consumption of iron rich wines (71) or drinking water (55).

The high intake of iron among the Bantu, amounting to 100 mg daily or more, is derived mainly from iron utensils used in cooking and in the preparation of kaffir beer (120) It has been calculated that a daily retention in the male of only 2 5 mg iron between the ages 20 and 40 years would lead to an accumulation of about 10 g iron by the age of 40 years (7) Other factors may, however, be of importance in producing the hemosiderosis which is commonly seen in the male Bantu, especially after the age of 40 years It has been suggested that the increased absorption might be a result of a widespread metabolic defect induced by chronic malnutrition (36, 39) However, the degree of siderosis is reported to be the same in apparently well nourished subjects (7, 53) Poor diet in animal experiments together with a low phosphorus content can, however, result in increased absorption of iron and massive iron storage (37) Even in animals on grossly deficient diets, an iron overload can largely be prevented by the addition of phosphates (51) suggesting that the excessive absorption in the animals is related more to the iron/phosphorus ratio of the diet than to an alteration of the intestinal mucosa caused by malnutrition The overall phosphorus content in the average Bantu diet is, however, if anything greater than normal (120)

It has also been considered feasible that a variety of unidentified toxic substances in alcoholic concoctions consumed by the Bantu in large amounts could together with malnutrition and hepatotoxic viruses, be responsible for potentiation of the liver damaging effects of large liver iron deposits (54)

The incidence of hemosiderosis in the Bantu identified by chemically determined liver iron exceeding 0 1 % of dry weight, has been reported to be 69 % in traumatic deaths (7) The incidence and severity tend to be distinctly lower in females than in males (10) The siderosis first becomes manifest in late adolescence and reaches its greatest severity after the age of 40 years The possible relationship between iron overload and tissue damage is subject to conflicting views (19, 71, 72) Some investigators have found cirrhosis of the liver in Bantu subjects with all grades of siderosis (39), while others (38, 54, 63) have found a close relationship between hemosiderosis and the more severe degrees of portal fibrosis or cirrhosis A combined chemical and histological study of 147 livers from persons who died accidentally showed portal fibrosis or cirrhosis in all cases where the iron concentration was above 2 % of dry weight (7) In another study (63) severe hepatic siderosis was present in 22 of 24 patients with cirrhosis, the average liver iron concentration for the whole group being 2 3 % of dry weight In the cirrhotic cases there were significant epithelial deposits in other organs making the overall histological picture virtually indistinguishable from idiopathic hemochromatosis Parenchymal deposits in other organs do not seem to occur unless cirrhosis is present (63), causing a widespread redistribution of iron Seven per cent of a randomly selected series of 100 diabetic Bantu subjects were diagnosed as having fully developed hemochromatosis, an incidence more than 40 times greater than that of an American series of diabetic subjects (100) The only finding which distinguished the Bantu hemochromatosis from the idiopathic type was the high iron concentration present also in the spleen (63)

Available evidence seems to indicate that the high iron intake *per se* is mainly responsible for the iron overload in the Bantu and that when excessive parenchymal deposits of iron are present for a long period, characteristic pathological and clinical features may develop Other factors such as malnutrition, alcoholism toxins and viral hepatitis may predispose to, or potentiate the effects

A Studies on bone marrow hemosiderin in relation to certain other hematological parameters and age

The most direct and practical method of assessing the iron stores is examination of fragments of bone marrow for stainable hemosiderin (8) The method appears to be a valid one since comparable results have been obtained when the non hemoglobin iron in marrow aspirates has been estimated chemically (30, 121) Weinfeld (121) found a highly significant correlation between the two methods For each histochemical group the mean of the chemical determination was significantly different from that of either of adjacent groups There was, however, considerable overlapping especially in the groups with higher hemosiderin scores He found no difference between smear technique and that of histological sections as regards the means of chemically determined non-hemin iron for the respective histochemical grades He also found a highly significant correlation between the iron stores in the liver and the bone marrow in basal subjects although the scatter of individual values was appreciable

It would thus seem that the bone marrow hemosiderin smear method should be satisfactory for screening purposes in the estimation of the reticuloendothelial iron stores, although it should be emphasized that this method gives no information on the ferritin part of the stores

The object of the present investigation was to study the bone marrow hemosiderin in relation to age and to certain other hematological parameters

Material and methods

Bone marrow aspirates were taken from 149 subjects of ages 3—65 years 13 were excluded — 7 because of technical failure to obtain enough marrow and 6 when found to have leprosy under treatment 136 healthy and apparently healthy subjects thus remained The children under 10 years (of both sexes) belonged to the Children s Home which is run by CNU The teenage boys who volunteered were inmates at the Home for Delinquent Boys* in Addis Ababa where CNU had made examinations during the course of a supplementary feeding program The adult men also volunteers were staff members of CNU and ESPC or maintenance workers at the ammunition factory in Addis Ababa* A few samples were also taken from patients who had recovered from minor illnesses at the Body Guard Hospital **

All were given a brief physical examination to rule out frank infections and other disorders From all except 5 venous blood was taken at the same time according to the technique described previously and always between 9 and 11 in the morning

All marrow punctures were made in the dorsal iliac spine a site which is psychologically preferable to the sternum particularly under the prevailing conditions (see above concerning traditional views of blood loss) After careful and reassuring information to each volunteer individually and following local anesthesia about 0 5—1 ml marrow was quickly aspirated with a bone marrow needle tightly connected to a 10 ml Luer Lok syringe The needle was rotated during the aspiration to ensure that enough marrow was obtained The marrow was ejected onto a concave glass and th marrow particles were moved up to the edges of the glass 6—8 slides with a thick film (squash preparation) were made from each aspirate

The conventional method of staining for hemosiderin was used The film is fixed in formaldehyde ethanol for 1—3 minutes (1 part 35 % formaldehyde (Merch p a) to 9 parts 95 % ethanol) after which it is washed in distilled water and placed for 5 minutes in 10 % potassium ferrocyanide (Merch p a) To this is added 1/2 volume 10 % iron free HCl and rapid mixing is performed by blowing air through a pipette into the solution The slide is kept there for 30 minutes after which it is washed in distilled water and dried All equipment used was carefully cleaned and washed in deionized water The potassium ferrocyanide was prepared fresh for each staining

The preparations were classified into six grades according to a modification of the method of Rath

---

* I am indebted to Ato Mebrato Yohannes Director of the Home for Delinquent Boys and to Colonel Alemayehu head of the ammunition factory for their kind cooperation and assistance

** I am grateful to Dr K Weithaler head of the Body Guard Hospital and his staff for permission to examine and test his patients and for their assistance

Table 13 *Number of subjects, mean age and age range in different hemosiderin grades*

Hemosiderin grade	0	trace	+	++	+++	++++	Total
No subjects	3	11	25	36	26	35	136
Mean age	4 3	12 7	18 0	19 7	26 2	35 5	
Range	3—6	3 5—20	3 5—40	9—65	7—55	16—60	

and Finch (93) 0 trace, + (slight) ++ (moderate) +++ (moderately heavy) and ++++ (heavy or very heavy)

They were examined and assayed independently by two examiners (M P* and Y H) who did not have access to each others results In 17 cases there was a divergence of one grade and in 2 of two grades (M P > Y H in 10 cases and Y H > M P in 9 cases) These were reviewed a second time and decision made regarding grouping In view of the fact that the method involves subjective evaluation the result must be considered satisfactory

Results

The number of subjects the mean age and age range in the different hemosiderin grades are given in Table 13

As is clearly demonstrated there was an increasing hemosiderin deposit with increasing age although with considerable overlapping

Fig 8 shows the percentage of grades +++ and ++++ in different age groups Here again it can be seen that with increasing age the number of subjects with an increased hemosiderin load in the bone marrow became higher In the age group 20—29 years slightly more than half the subjects (52 8 %) were given a score of +++ or ++++, whilst about four fifths (81 6 %) of those ≧30 years of age were given this score

In no case below 10 years of age was a score of ++++ noted In the age group 10—19 years this score was recorded in 2 8 % in the group 20—29 years in 36 1 % and in the group ≧30 years in 55 3 %

Chronic longstanding infections may result in increased hemosiderin deposits in the reticuloendothelial cells in the bone marrow (93) In order to minimize the possibility that the increased hemosiderin deposits found in increasing percentage with increasing age were caused to a considerable degree by this mechanism all subjects with an ESR of more than 20 mm were excluded The result is seen in Table 14 As will be seen these percentage figures are very close to those in Fig 8, where all subjects are included

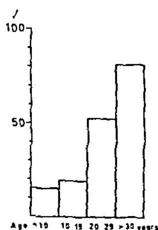


Fig 8 Percentage of subjects with hemosiderin grades +++ and ++++ (scale 0 — ++++) in different age groups

---

* I am very grateful to Mrs Margit Persson S R N who has had long experience in bone marrow hemosiderin evaluations for her kind and generous assistance

Table 14 *Percentage of subjects with increased hemosiderin load (grades +++ and ++++) and ESR ≦20 mm*

	<10 years	10—19 years	20—29 years	≧30 years
Number	17	24	35	30
% with grades +++ and ++++	17.6	20.8	54.3	80.0

Correlation between hemosiderin load and certain other hematological parameters

The means and SD of Hb, PCV, SI, TIBC and % transferrin saturation in the different hemosiderin grades are given in Table 15.

The mean Hb values were lower in the lower hemosiderin grades, reflecting the fact that these grades included subjects aged less than 15 years. The mean PCV values parallelled the Hb values making the mean MCHC agree well with that presented above for healthy and apparently healthy subjects in Addis Ababa (Chapter III). On the other hand the mean value for ESR gradually diminished in the higher hemosiderin grades. This reflects the higher prevalence of raised ESR in younger subjects which has also been discussed previously.

20 subjects were anemic according to previously mentioned WHO criteria. 14 of those (70.0 %) were teenage boys from the Home for Delinquent Boys. All were marginally low. ESR was above 20 mm in 10 of those 20 anemic subjects as against 13.0 % in the non anemic.

The mean serum iron values in hemosiderin grades +++ and ++++ were higher than in the lower hemosiderin grades. The differences between the mean value in grade ++++ and those in grades 0 — trace + and ++ were highly significant while that between grades ++++ and +++ was not significant.

The mean values for TIBC showed only minor fluctuations in groups + — ++++, while the value for group 0 trace was slightly although not significantly higher.

Consequently, the transferrin saturation was higher in the groups +++ and ++++, reaching a value of 49.8 % in group ++++.

Table 15 *Hematological values in different hemosiderin grades*

Hemosiderin grade		Hb	PCV	ESR	SI	TIBC	% satur
0 trace	Mean	13.8	43.5	17.9	70	309	23.0
	S.D.	± 1.64	± 5.53	±12.86	± 35	± 59	±12.2
	No	14	14	14	11	11	11
+	Mean	14.7	47.3	14.0	86	283	30.8
	S.D.	± 1.21	± 4.13	±10.38	± 37	± 43	±12.6
	No	24	24	24	22	22	22
++	Mean	14.8	47.3	13.6	82	295	28.4
	S.D.	± 1.52	± 4.12	± 9.65	± 37	± 35	±12.0
	No	35	35	34	32	31	31
+++	Mean	15.0	48.3	12.6	110	284	38.4
	S.D.	± 1.45	± 4.47	± 9.84	± 39	± 32	±12.5
	No	24	22	24	22	22	22
++++	Mean	15.5	50.9	8.8	140	284	49.8
	S.D.	± 0.99	± 3.44	± 6.21	± 62	± 35	±22.7
	No	32	32	33	33	33	33

Table 16 *Hematological values in subjects ≥20 years with ESR ≤20 mm in different hemosiderin groups*

Hemosiderin grade		Hb	SI	TIBC	% satur
Trace — ++	Mean	15 8	110	297	37 1
	S D	± 1 26	± 33	± 29	± 11 0
	No	22	20	20	20
+++	Mean	15 7	121	289	41 5
	S D	± 1 40	± 34	± 33	± 9 9
	No	13	13	13	13
++++	Mean	15 6	145	282	52 2
	S D	± 0 92	± 61	± 35	± 22 1
	No	29	30	30	30

As it is obvious that the mean values of several parameters are influenced by age and thereby also, as has been discussed earlier, by possible subclinical infections as judged from raised sedimentation rates those with an ESR higher than 20 mm (24 subjects) and also the subjects below 20 years of age were excluded In 6 cases the ESR was not recorded These were also excluded The result is seen in Table 16

As can be seen the mean values for SI and transferrin saturation *gradually increased* with increasing hemosiderin deposits while the mean TIBC remained at about the same level The difference in SI between the lowest and highest hemosiderin group was almost significant The mean Hb was similar for all groups and agreed with the mean for adult healthy and apparently healthy males (see Chapter III) 30 subjects (46 2 %) had a hemosiderin grading of ++++

Comments

Absence of stainable hemosiderin in the bone marrow was noted in only 3 cases (2 2 %) These were all young (3—6 years) In all the others it was present and in 44 9 % there was an increased load (+++ and ++++) Such a heavy hemosiderin load was noted even at early ages the youngest subject with a score of +++ was 7 years old and the youngest with a score of ++++ was 16 years Generally there was an increasing hemosiderin load with increasing age, +++ and ++++ being noted in 81 6 % of those ≥30 years All the subjects were medically fit and not suffering from any longstanding chronic infections, although 18 5 % had an ESR above 20 mm Even after these had been excluded the same relative percentage of increased load persisted in all ages

Increased bone marrow iron deposits may occur principally when there is a shift of hemoglobin iron into tissues, as occurs in any *anemia not due to blood loss or to insufficient* iron intake when there is impaired release from the reticuloendothelial system, as occurs in infection, or when there is an absolute increase in storage iron (8) It is highly unlikely that any of the first two mentioned mechanisms should be in operation It is however, quite conceivable that there was an absolute increase in the body storage iron When the stores are increased there is usually a corresponding increase in marrow hemosiderin but discrepancies do occur, since iron at this site is only a reflection of reticuloendothelial deposits and is not indicative of the amounts of parenchymal iron present (8, 49)

No less than 46 2 % of subjects 20 years or more with ESR ≤20 mm had a hemosiderin score of ++++ (Table 16) This is a totally different distribution from that reported for healthy adults in the United States and Sweden (8 48) Hansen and Weinfeld (48) found in 26 hematologically normal

males with an average age of 59 years, that with a hemosiderin scale of 0 to +++, 4 were scored 0, 19 + and 3 ++ None were graded +++ Weinfeld (121), however, in 15 control male found a significant correlation between total non-hemin iron in the marrow and age, although this was due mainly to a rise in the lower ages

The finding of increasing serum iron with increasing hemosiderin deposits is in agreement with the findings in conditions of iron overload (8) It was also found in Bantu subjects that there was a stepwise increase in the mean serum iron with increasing severity of hepatic siderosis (50) These increases in mean serum iron were greater and statistically more significant in the Bantu patients without infections However, Hathorn *et al* (50) also found a significant elevation of mean TIBC, once appreciable hepatic siderosis was present This is contrary to the findings in the present study where the mean TIBC remained at about the same level in all hemosiderin groups (Tables 15 and 16)

B Evaluation of the iron stores by desferrioxamine

During the past few years desferrioxamine (DF), capable of chelating body iron, has been used extensively (25 26, 43, 44, 45, 49) Studies made by Hallberg *et al* (43, 44) indicate that the iron excreted with DF does not originate from iron bound to transferrin and that it does not appear to be related to the red cell catabolism On the other hand Hallberg *et al* (45) found a very high correlation between the increase of urinary iron excretion after administration of DF and the concentration of total non-hemin iron in the liver Harker *et al* (49) also found a good correlation between chelate iron excretion and liver parenchymal iron, but not reticuloendothelial iron It was possible to dissociate these storage sites and to show that patients with only reticuloendothelial iron overload and high serum iron saturation did not excrete a significant amount of chelate iron The results were considered to indicate that DF enters liver parenchymal cells, where it binds excess storage iron About two-thirds of the intracellularly formed ferrioxamine passes into the plasma to be excreted via the kidney into the urine and about one-third is excreted by way of the bile into the feces

In the present study an attempt was made to obtain information, by means of the DF test, regarding the magnitude of the body iron stores in male subjects in whom bone marrow hemosiderin was also studied

Material and methods

19 apparently healthy Ethiopian male subjects aged 20—52 years had a bone marrow aspirate stained for hemosiderin as described earlier All 19 were also included in the bone marrow study presented above Determinations of Hb PCV ESR SI and TIBC were made according to methods described in Chapter III Nine of the subjects collected a 24 hour urine specimen about 1 week prior to the DF test In 7 cases this collection was complete At 8 a m an intramuscular injection of 500 mg Desferral® (CIBA) dissolved in 10 ml distilled water was given after the subjects had emptied the bladder The urine was then collected during the next 6 hours only as it was not considered feasible to obtain a complete 24 hour specimen The urine samples were collected in polyethylene bottles which had been carefully washed and rinsed with deionized water

The urine specimens thus collected were analysed for iron according to a method described by Hallberg *et al* (43)

Results

The hemoglobin concentrations ranged between 14 7 and 17 5 g % (mean 16 2±0 95 g %) and thus none of the subjects were anemic according to previously discussed criteria (in one case the Hb was not recorded but the PCV value was 52 %, not suggestive of anemia) The mean PCV and MCHC values were also very close to those reported previously for adult healthy and apparently healthy males in Addis Ababa (Chapter III) All had an ESR of 20 mm or less All the subjects had stainable hemosiderin in the

bone marrow — two had a 'trace', one +, one ++, three +++ and twelve ++++ (see grading on page 38)

In the group with high hemosiderin scores (+++ and ++++) the mean SI was higher (162±60 µg %) and the TIBC lower (284±31 µg %) than in the group with low scores (SI 109±26 µg % and TIBC 304±42 µg %) These differences were not significant however

The iron excretion in the 7 subjects from whom a complete 24 hour urine specimen was obtained before the DF test ranged between 0 00 and 0 13 mg

The mean value for the 6-hour iron excretion after DF in the high hemosiderin group was 0 50±0 307 mg, and was not significantly higher than that in the low hemosiderin group, 0 32±0 045 mg

In the high hemosiderin group the total 6-hour iron excretion ranged from 0 18—1 43 mg and in the low group from 0 26—0 37 mg The median values in these groups were 0 42 and 0 32 mg respectively The highest excretion value, 1 43 mg/6 hours, was reached by a 44-year old healthy man He had an SI value of 307 µg %, a transferrin saturation of 90 6 %, and a hemosiderin score of ++++ The lowest excretion, 0 18 mg was noted in a 50-year old man, also with a score of ++++ His SI value was 168 µg % and transferrin saturation 62 0 % See Fig 9

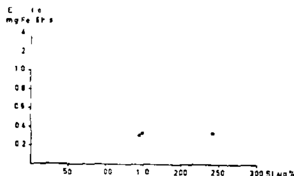


Fig 9 Iron excretion after 500 mg DF in relation to serum iron in 19 males 4 with hemosiderin grades trace — ++ (X) and 15 with grades +++ and ++++ (o)

The amount of urine voided was quite low in several cases (hot season) Two subjects with the lowest iron excretions in the high hemosiderin group voided only 168 and 190 ml, respectively One man misunderstood the instructions and manged to drink 3 liters, voiding 1,640 ml (iron excretion 0 34 mg) The others voided between 145 and 880 ml The iron excretion per ml voided urine in the low hemosiderin group was 0 92 µg and in the high group 1 47 µg The difference is not significant

Comments

In normal male subjects the mean 24-hour iron excretion after administration of DF in a dose of either 500 mg or 10 mg per kg body weight has been reported to be between 0 59 and 0 72 mg (6, 43, 44, 45, 49) The *maximal excretion seems to occur 3—4 hours* after the DF injection (43), the mean excretion during the first 6 hours corresponds approximately to half the total 24 hour excretion (6, 43), and has been reported to be between 0 29 and 0 40 mg (6, 43, 96) in normal males In hemochromatosis, iron excretion values of above 20 mg in 24 hours have been reported (49) Rosen *et al* (96) *reported values ranging between 1 2 and 7 0* mg in 6-hour urine specimens in patients with hemochromatosis In transfusional hemosiderosis a 24 hour excretion of about 10 mg iron has been found (49)

The iron excretion in the subjects with low — normal hemosiderin scores (trace — ++) in the present series lay entirely within reported normal ranges However, the excretion in the subjects with high hemosiderin scores (+++ and ++++) was not impressive In 9 of the 15 males a value of 0 40 mg or more was noted and in only one case did it exceed 1 mg (1 43 mg)

Several subjects had a low diuresis probably due to the rather high environmental temperature and some reduction in iron excretion has been reported with fluid restriction (70),

although this mechanism can hardly account for a considerably reduced excretion

In evaluation of the results of the DF test the following seems relevant The reticuloendothelial cell may be regarded as the normal regulator of serum iron and the normal site of iron stores (49) Any influx of excess iron is compensated for by a corresponding increase in reticuloendothelial iron but in some iron overload states for reasons that are not entirely clear, regulation by the reticuloendothelial cells is inadequate in which case serum iron rises and parenchymal iron deposition occurs (49)

In idopathic hemochromatosis the iron deposits in the RES cells are not prominent while in the iron overloaded Bantu they can reach considerable proportions (8), the emphasis with increasing liver iron being on reticuloendothelial involvement (19)

The state of the liver parenchymal iron stores in the subjects under study was not known, although there may be reason to believe that they were probably not excessive (see below) The mean SI in the high and low hemosiderin groups did not differ significantly although the latter group comprised only 4 subjects There was however a highly significant difference in mean SI between the high hemosiderin group in this DF study and the low (trace — ++) group with 20 subjects in the bone marrow study (see Table 16), while the mean TIBC was about *the same in both these groups* The raised SI and transferrin saturation in the high hemosiderin group and the slightly although not significantly higher urinary iron excretion after DF might be suggestive of an incipient parenchymal cell overload (49)

Harker *et al* (49) postulate in their study that iron for both urinary and fecal excretion mobilized by DF is derived from the iron deposits within liver parenchymal cells and conclude that the DF test is useful for detecting significant liver parenchymal iron overload If this is accepted and applied to the results in the present study the conclusion is that increased hemosiderin deposits found in the bone marrow represent a mainly reticuloendothelial iron overload state in the body

C Liver iron in autopsy material

As mentioned previously the iron content of the Ethiopian diet is often extremely high in adults in the order of 300—500 mg/day The situation is thus similar in this respect to that in the Bantu population in Southern Africa, although the availability of the iron in the Ethiopian diet may be much lower than that of the Bantus The absorption of iron from Bantu beer has been found equal to that of a ferric salt (12)

The studies of hemosiderin in the bone marrow indicated an increasing reticuloendothelial load with increasing age — for the age group ≧30 years definitely above the Western standard It thus seemed of interest to study by chemical methods the iron content of the livers of Ethiopian subjects

As was mentioned earlier (see 'Introduction to this Chapter), several studies among Bantus have revealed a high incidence of siderosis in autopsy or biopsy material Bothwell *et al* (7) found in an analysis of 147 Bantu subjects, aged 11—70 years (131 of them males), who died from accidents that 89 % had varying degrees of siderosis (liver iron ≧100 mg/100 g *dry weight) In 48 %* the siderosis was of a mild degree (100—390 mg/100 g) but in 19 % the liver iron concentration exceeded 1,000 mg/100 g The remaining siderotic cases showed a moderate increase (400—990 mg/100 g) and only 11 % had values below 100 mg/100 g

In a recent report by Weinfeld *et al* (122) 27 Swedish control males aged 24—63 years had in biopsied liver material a mean value for non hemin iron of 80 2 mg/100 g dry weight (range 19 4—227 0) Seven of the subjects or 26 % had iron values above 100

mg The corresponding value for 16 women was 37 1 mg (range 5 5—106 6)

Material and methods

Autopsies are made in Addis Ababa mainly on medicolegal indications Through the courtesy of Dr E Codeleoncini at the Department of Pathology at the Menelik Hospital liver specimens were obtained from 107 subjects representing consecutive autopsies in June and October 1967 Nine specimens had to be discarded and 98 thus remained for analysis (92 males and 6 females) A total of 76 (70 males and 6 females) had died through road accidents (31) criminal assault (23) suicide (hanging) (9) or suffocation drowning and similar accidents (13) * No information on previous diseases or nutritional status was available Two of the persons who died through hanging showed concomitant diseases 1 (alcoholic?) cirrhosis and 1 exudative pleuritis They were nevertheless included among the accidental and violent deaths The main causes of death among the 22 who had died of disease was heart failure (9) infections (6) cerebral hemorrhage (3) internal bleedings (2) pulmonary cancer (1) and uremia (1)

The liver specimens were stored deepfrozen until analysed for nonhemin iron according to a method described by Weinfeld (121) The analyses were performed at Dr A Weinfeld's laboratory at Gothenburg under his supervision The results are expressed as mg non hemin iron/100 g dry liver weight

Results

The results for males are summarized in Table 17 The age of the males who died from trauma ranged between 8 and 70 years The highest mean value for liver iron was found in the age group 20—29 years and the lowest in the 30—39 year group (almost significant difference) The mean value in the groups <20 years and ≧40 years did not differ significantly *The mean value for all subjects* ≧20 years and who died from trauma did not differ significantly from that in the subjects who died from disease (age range 20—70 years)

In subjects ≧20 years who died from trauma the nonhemin iron ranged from 8 2—266 0 mg/100 g and 26 out of 65 subjects, or 40 0 %, had values ≧100 mg In the male subjects who died of disease the range was 13 1—213 3 mg/100 g and 8 subjects or 36 4 % showed values exceeding 100 mg

All 6 females had died through accidents *Their ages ranged between 3 months and 70* years and the non hemin liver iron ranged from 22 9—103 9 mg/100 g

Table 17 *Liver iron (mg Fe/100 g dry liver weight) in males who died from trauma and in males who died of disease in different age groups*

Age	Mean and SD	<100 mg No	%	100—199 mg No	%	200—299 mg No	%	Total number
				Died from trauma				
<20 years	79 8 ±55 48	4	80 0	1	20 0	0	—	5
20—29 years	105 4 ±72 30	17	54 8	10	32 3	4	12 9	31
30—39 years	62 7 ±40 55	14	70 0	6	30 0	0	—	20
≧40 years	94 4 ±59 26	8	57 1	5	35 7	1	7 1	14
≧20 years	[illegible] ±63 26	39	60 0	21	32 3	5	7 7	65
				Died of disease				
≧20 years	[illegible] ±54 56	14	63 6	7	31 8	1	4 6	22

* These 76 subjects will be referred to in the following as subjects who died from trauma

The mean values and r ı hemin iron in male subjects w r o rauma are close to those reportc ł (121 122) for control Swedı h t otal of 40 % of the males n rauma had non hemin iron v 100 g which is higher than ı Wein feld *et al* (122), 26 ° lower than in the series of Bo h b adlow (7) of Bantu males ı r o had values of 100 mg o ady of Mayet and Bothwell fro ı (75) on 738 livers obtain ı 554 of which were from whit d Indian subjects who had d ec urra the median values in all r ı 281 and 60 mg/100 g respect v ı ed to dry weight assuming a wat c t of 70 % (121)) The correspond n r ed an values for females were 50 59 and 39 mg 100 g respectively In 708 au ops ed adults from Ireland Israel Japan South Africa (white subjects), Boston and San Francisco Mac Donald and Pechet (73) found that 16 % had liver iron values of more than 100 mg/100 g dry weight

No information on previous diseases was available for the subjects under study and nothing was known about their food habits and iron intake It is probable however that a significant number came from the poorer strata of the society with poor eating habits although no autopsy records were made of gross malnutrition intestinal helminths or signs of anemia No fewer than 32 of the males or 46 % had committed suicide or were victims of criminal assault There is therefore reason to believe that this series is not comparable as regards health standard and possibly iron intake with the adult healthy and apparently healthy males from whom bone marrow aspirates were taken

Although it would have been expected to find a higher frequency of high values the number of low values, on the other hand, was small Weinfeld (121) found a mean value of 5 5 mg/100 g (range 2 7—10 0) in 6 male subjects with iron deficiency anemia In the present material only one male, 40 years old and killed in a road accident came below 10 mg (8 2) Of the females 2 or possibly 3 were at the menstruating age and showed values ranging from 22 9—53 2 mg/100 g which were well within the range found in menstruating Swedish women (121, 122)

Although the present series is not satisfactory as regards background data of the subjects and is probably not comparable to that in the bone marrow hemosiderin study, it could be concluded that the non hemin iron values in the liver specimens were comparable to those in a normal Swedish control material supporting the view of an adequate but not excessive uptake and retention of dietary iron Obviously then the extremely high amounts of iron in the Ethiopian diet are available for absorption only to a limited extent (cf Chapter II)

Summary

From 136 healthy and apparently healthy subjects aged 3—65 years, bone marrow smears were examined for stainable hemosiderin, which was graded from 0 to ++++. The results indicated that with increasing age the percentage of subjects with increased (+++ and ++++) hemosiderin load rose successively. In ages $\geq$30 years about 80 % had +++ and ++++ hemosiderin. Only 3 young children had no detectable hemosiderin in the marrow.

In subjects $\geq$20 years with ESR $\leq$20 mm there was a successive rise in SI with increasing hemosiderin grades, with an almost significant difference between the lowest and highest hemosiderin groups, parallelled by a rise in transferrin saturation.

In 19 adult male Ethiopians a desferrioxamine (DF) test was made in which 500 mg DF was given intramuscularly. Four of these subjects had hemosiderin grades trace — ++, and 15 had grades +++ to ++++. The mean values for 6 hour urine iron excretion were 0.32 and 0.50 mg, respectively, a non-significant difference. There was considerable overlapping between the excretion in the two groups and the two extreme values 0.18 and 1.43 mg were both reached by older men with a hemosiderin score of ++++ and high serum iron and transferrin saturation values.

For reasons discussed it is suggested that the increased iron deposits found in the bone marrow may have represented a mainly reticuloendothelial iron overload state in the body.

98 autopsy liver specimens were analysed for non hemin iron. 76 were from persons who died from accidents or violence (70 males and 6 females) and 22 from subjects who died from disease (all males). The mean value for non hemin iron was very similar in both these groups (for males $\geq$20 years 88 and 86 mg/100 g dry liver weight, respectively) and corresponded to that found in normal Swedish males.

Among all males $\geq$20 years 39 % had values $\geq$100 mg/100 g, the highest value being 266 mg, while only 1 was below 10 mg.

There was a high incidence of suicide and homicide in the series and there seems reason to believe that it cannot be compared to the healthy and apparently healthy males in whom marrow hemosiderin was studied.

Evidence presented supports the view of an adequate or more than adequate but not excessive uptake and retention of dietary iron and that the extremely high amounts of iron in the Ethiopian diet thus seemingly are available for absorption only to a limited extent.

CHAPTER V

Hematological investigations in a representative Ethiopian highland village

Tropical anemias*

There is abundant evidence that anemia is present among large sectors of the population in tropical countries In many areas and especially among children and women of fertile age it may reach appalling degrees and frequencies (104 107) Etiologically, dietary deficiencies infections and parasitic infestations are by far the most common causative factors (62), although in certain areas anemias of other causes such as sickle cell anemia or other hemoglobinopathies are prevalent (123)

Although deficiencies of several essential nutrients result in anemia those deserving primary consideration are deficiencies in iron vitamin B 12, and folic acid (89) The same type of anemia may result from dietary deficiency *per se* poor absorption and utilization and/or increased requirements such as occur in rapid growth and in pregnancy Associated parasitism may enhance the significance of one or more of these factors (89)

The morphological picture of a fully developed iron deficiency anemia is characterized by hypochromic and microcytic erythrocytes low levels of serum iron, raised transferrin levels with a low transferrin saturation and absence of stainable hemosiderin in the bone marrow The typical blood picture in anemia caused by deficiency of vitamin B 12 or folic acid is one of macrocytosis with a megaloblastic bone marrow The term dimorphic has been proposed to refer to cases in which both macrocytic and hypochromic microcytic anemia exist at the same time as a result of mixed deficiency (Trowell, quoted by (123))

Studies carried out in India Africa and Central and South America have shown that anemia due to iron deficiency is the most common in these regions (62) Although in most tropical countries the dietary iron intake may be equal to or greater than that accepted for adequate diets in North America and Europe (62, 88) several factors may interfere with the absorption The diets are generally rich in bulky carbohydrate foods from which iron absorption is low (58 80) and may have high contents of phosphates and phytates with low calcium levels (23) which may interfere with the iron absorption although the views are conflicting as to whether or to what extent this interference plays a role (8)

Important factors in the development of iron deficiency anemia in females are repeated pregnancies and lactation, when the demands for nutrients exceed those required to maintain health in a non pregnant woman, and where high frequencies of such anemia have been reported (89, 107)

Some controversy exists as regards increased losses of iron through the skin by sweating in hot climates (8) There seem to be indications that under adverse temperature conditions the basal loss of about 0 2 mg per day may be increased to 0 5 mg (9)

The association of hookworm infection and anemia has long been recognized, although apparently there has to be a certain worm load, *as measured by the number of eggs* per gram feces, before a significant decrease of the hemoglobin concentration is achieved This critical level was found to be 5 000 eggs

* This review does not pretend to cover the enormous literature dealing with this subject but to serve mainly as an introduction to this chapter

for adult men and 2,000 for children and women (68), reflecting the greater vulnerability in the latter groups where growth and an increased physiologic iron drain raise the demand for iron 5,000 eggs per gram feces correspond to about 250 worms, meaning a loss per day of about 10 ml blood, containing about 5 mg iron out of which 3 mg would be lost in the feces after re absorption of the rest (68) There are great individual variations however An important factor determining the development of anemia is the dietary iron content (29) and it has been demonstrated that hookworm anemia can be controlled by iron without deworming (35)

Hookworms are among the most important causes of ill health in many developing countries and constitute a formidable public health problem (29)

Nutritional megaloblastic anemias in the tropics are comparatively much less frequent than iron deficiency anemias Both sexes may be affected but women predominate and these anemias are far more common in pregnant than in non pregnant women (89) Most of them respond to treatment with folic acid and some to vitamin B 12 (88) Studies in India in pregnant women showed that although the majority of the anemia cases were due to iron deficiency there were indications that 25—40 % may have had concomitant deficiency of folic acid and/or vitamin B 12 (89)

Vegetable foodstuffs do not contain vitamin B 12 and in vegetarians this vitamin is presumably obtained through activity of intestinal micro organisms (88) Folic acid is present in appreciable quantities in green leafy vegetables legumes and cereals, any cereal diet would give an adequate daily intake (88) and it remains obscure which other factors may be operative in producing folic acid deficiency anemias Cooking losses increased demands during pregnancy and malabsorption have been suggested causes (88)

Protein—calorie malnutrition is exceedingly common among infants and pre school children in developing countries, and the most severe form, kwashiorkor, is usually combined with a mild to moderate anemia, also in the absence of other obvious anemia causes (65) Anemia has also been described in cases of cystic fibrosis with protein failure, without other causes of anemia, and responding to high protein feeding before the addition of pancreatic enzymes (102) Animal experiments (46, 105) with low protein diets resulted in rats (46), in a slight fall in the hemoglobin concentration but a large fall in total hemoglobin, the anemia being microcytic and normochromic or slightly hypochromic, while in the Rhesus monkey (105) a moderate anemia of the normocytic and normochromic type developed Doubts regarding the part played by protein malnutrition in the production of anemia have arisen as a result of the poor hematological response which commonly follows the administration of protein to patients with kwashiorkor or marasmus The anemia is ascribed to diminished erythroid activity in the bone marrow (3, 103) which has been interpreted as an adaptation to undernutrition (103) The role of moderate protein malnutrition in the production of anemia is, however, not clear (88)

A mild non-progressive anemia frequently accompanies various chronic infections and systemic diseases, usually normocytic and normochromic but sometimes hypochromic or even microcytic (17, 123) The hypochromia is usually not as marked as in iron deficiency anemia and the reticuloendothelial iron in the bone marrow is normal or increased The serum iron is low and the transferrin levels decreased The anemia is caused by a modest shortening of the erythrocyte survival time for which the bone marrow fails to compensate by increased production (17) Except for severe acute infections, especially in small children infections of less than a month s duration are not, as a rule accompanied by significant anemia and it has been

suggested that the previous nutritional status of the patient may possibly be of some importance in determining whether or not anemia develops (18). *The interaction of nutrition and infection* has been well documented (99). Although a variety of viral and bacterial infections are exceedingly common in tropical countries the exact role played by the infection in producing anemia has not been satisfactorily demonstrated. Increased sedimentation rates are often noted in tropical Africa (113) and are usually attributed to malnutrition and infection. When in apparently healthy Bantu males those with an ESR above 8 mm were excluded the hemoglobin levels of the remainder were similar to those of Europeans with the same low ESR (113). In this connection it might be appropriate to point out that optimum hematological values in tropical and temperate zones are the same (62) and that no racial differences occur although other opinions have been expressed (20).

Malarial infection of a slight degree may not cause anemia. Longstanding untreated infections cause a hemolytic anemia which is more severe in malignant tertian malaria. In uncomplicated cases the response to treatment is extremely rapid. In hyperendemic regions, children have a rising parasitic count from birth to the second year, coinciding with the time when the immunity is lowest, after which there is a fall (113).

The hemoglobinopathies of public health *importance are those characterized by hemoglobins* S, C and E, and the thalassemias. The gene for Hb-S is widely distributed in equatorial Africa but limited to the north by Sahara and the Ethiopian highlands (52). Hb-C is found in West Africa, while Hb-E is seen primarily in the tropical regions of South-East Asia, and the thalassemias have their greatest known prevalence in the Mediterranean regions and in South-East Asia (52).

The hemoglobinopathies caused by Hb-S, Hb-C and Hb-E in the homozygous form give rise to hemolytic anemias, the first mentioned sickle cell anemia, presenting the most severe forms. In the heterozygous state the *sickle cell trait may present symptoms under* certain circumstances while the other two have no known clinical effects (52).

The village Ijaji

In the beginning of 1963 longitudinal studies of children 0—10 years old started in the village Ijaji 220 km west of Addis Ababa (77). The village was chosen after careful surveying and is considered typical of and representative for the living conditions in the Ethiopian highlands. It had at that time about 1 600 inhabitants, but has since grown considerably. In October 1966 when the last census was made it had 2,100 inhabitants. The size was therefore considered suitable for nutritional studies.



Fig. 10. The main road through Ijaji.

Approximately 25 % of the heads of households are farmers and 25 % traders, a reflection of the fact that the village is located along a main road in an agricultural region. About 20 % are bar-owners or subsist by producing beer or local liquors, most of these are women.

80 % are Orthodox Christians and the remainder Moslems. The predominant ethnic group is the Galla tribe (60 %), followed by the Amhara (30 %). A little more than 10 % of the families are polygamous. In

1966 when the most comprehensive census was made 43 % of the population were below 15 years 54 % were females with the highest predominance in the ages 20—40 years (56)

In the farming areas of the village the typical house is a circular hut with a thatched roof Several such huts are gathered in fenced compounds The huts have an earthen floor and no windows, and usually have a fire-place on the floor in the middle of the hut Chicken calves and other animals are not seldom kept in the living houses

The houses along the road and around the market place are usually of better standard they are rectangular and most often have a roof of corrugated iron

Water is available all the year round from two covered wells and from three streams

In 1962 a health station was started mainly concerned with curative work The nearest hospital is 90 km away

An elementary school started in 1962 From a modest start the number of pupils has grown and in 1967 was about 250 in grades 1—6 It can therefore be expected that the degree of literacy will increase In the census of 1966 75 % of the heads of households and 98 % of the wives were illiterate

Material and methods

In November 1965 433 children of ages 0—11 (—12) years were clinically examined in Ijaji to obtain a base line material before the start of an applied nutrition program These corresponded to approximately 75 % of all children in this age group in the village 402 children or 92 8 % were tested hematologically which must be considered very satisfactory under the prevailing conditions A stool sample was obtained from 346 children 304 of these were tested hematologically

In December 1965 100 school children aged 4—20 years were examined in a small village Backo 45 km west of Ijaji This village greatly resembles Ijaji and is located at the same altitude The school had started a few months earlier in very primitive premises and comprised grades 1 and 2 The children can be considered representative of the village and all children in the school were examined all had a blood sample taken and most of them also had urine and stool analysis 52 of these 100 children aged 10—14 years were included in the present study to obtain full age continuity 45 children tested hematologically in Ijaji were 10—12 years These are included in the 10—14 year group unless stated otherwise

Thus the study concerns a total of 454 children aged 0—14 years Up to 10 years there was about the same number in each sex (177 boys and 180 girls) In the 10—14 year group boys predominated (69 boys and 28 girls)

In November 1967 416 persons aged 15 years and older were called for examination in Ijaji according to a predecided geographical sampling system to ensure that all areas and thereby the main professions would be equally represented (farmers and traders) 271 or 65 1 % attended and had an abbreviated clinical examination Of these 196 or 72 3 % were tested hematologically 15 persons refused blood test and for the remainder there was no laboratory capacity

During the past few years when longitudinal studies were in process the farmers had been on the whole co-operative while certain trading areas had shown some resistance The fact that about 75 % of the children who were called for examination attended means however that a very good representativity was achieved The precise figure cannot be given as the census was made 2 months earlier and the mobility is very high As for the adults the distribution of the clinically examined persons regarding sex and religion corresponded closely to that found in the last census made during the fall of 1966 — 44 % of the subjects above 15 years were males and 81 % (of heads of households) were Orthodox Christians the remainder Moslems In the clinically examined material there was however an over-representation of farmers (46 %) compared to the census (28 % of heads of households in the census) indicating a poorer attendance of people belonging to other occupational categories

The attendance figure of 65 1 % of the adults has to be considered satisfactory especially in view of the fact that it was announced that blood testing was to be done although on a voluntary basis

Capillary blood for determination of hemoglobin packed cell volume and sedimentation rate was taken from all children referred to above after previous warming and cleaning of the hands in warm water The determinations were made according to methods described previously

The subjects aged 15 years and older had venous blood taken by means of a Vacutainer according to a previously described technique Unfortunately 60 blood samples for Hb PCV and ESR from adults had to be discarded because of a technical mishap

Blood for determination of serum iron and transferrin was taken from 117 adults at the same time (although in different tubes) according to a previously described technique and always between 9 and 11 a m These 117 persons represented all cases from whom blood samples were taken between 9 and 11 a m After clotting the samples were spun and the serum separated and kept in a field deep freezer for 1—2 days before being taken to Addis Ababa for processing

From 17 females and 1 male (= all attending and agreeing to blood tests on one particular afternoon) blood was taken for determination (on serum) of folic acid and vitamin B 12 This serum was kept deep frozen for about 1 month after which it was processed at the Institute of Medical Chemistry in Uppsala Sweden

81 stool samples were obtained fram the 271 adults who had a clinical examination

In May 1967 30 blood samples for serum iron and transferrin determinations (and at the same time for Hb PCV and ESR) were obtained from children ≥5 years in Ijaji at a follow up examination there and 46 blood samples for the same purpose from Backo school children aged 5—14 years All these samples were taken by means of Vacutainers (also for Hb PCV and ESR) using the same technique as described earlier and with the same time precautions (9—11 a m) The 30 samples from Ijaji represent all children attending the last morning of examination and the 46 from Backo all children examined at that time in the school in this age group

On all blood tested subjects a thick blood film was examined for malaria (This was negative in all cases)

———

For the sake of simplification the subjects from Ijaji and Backo are referred to in the following as from Ijaji only

Results

The mean values and SD of the determinations of Hb, PCV, MCHC and ESR are presented in Table 18, and the relative distributions of Hb PCV and MCHC in Figs 11, 12 and 13 (infants excluded) For comparison the corresponding mean values from the normal material in Addis Ababa are given

The lower altitude in Ijaji (1 850 m) would justify a deduction of about 0 2—0 3 g % from the mean Hb values in Addis Ababa to make them comparable to the mean values in Ijaji (see previous discussion on this subject) When this deduction was made there was still a marked difference in mean Hb values between the normal material in Addis Ababa and those from Ijaji in all age groups The mean PCV values were slightly less depressed resulting in a lower mean MCHC in the village population compared to Addis Ababa The lowest mean MCHC value was recorded in the age group 6—11 months It should also be noted that in all age groups there was a considerable difference *in mean* ESR *compared* to the Addis Ababa material

In adult males the highest mean Hb value was recorded in the ages 25—44 years (14 2 g % n = 35) and the lowest in the group ≥45 years (12 9 g % n = 13) In the group 15—24 years the mean Hb was intermediate (13 2 g % n = 8)

In adult non pregnant females there was a successive decrease with increasing age 15—24 years (n = 11) 13 2 g % 25—34 years (n = 22) 12 6 g % 35—44 years (n = 17) 12 6 g % and ≥45 years (n = 16) 12 1 g %

Table 18 *Hematological values in different age groups in Ijaji For comparison mean values from the healthy and apparently healthy subjects in Addis Ababa (cf Tables 4 and 8)*

		Ijaji				Addis Ababa			
Age group		Hb	PCV	MCHC	ESR	Hb	PCV	MCHC	ESR
1—5 12	Mean	11 3	37 8	29 6	28 2	—	—	—	—
	SD	± 1 04	± 3 40	± 1 86	± 10 86				
	No	11	10	10	11				
6—11 12	Mean	10 1	36 7	27 4	25 9	—	—	—	—
	SD	+ 1 54	± 3 25	± 2 31	± 14 13				
	No	20	22	20	22				
1—4	Mean	11 1	37 9	29 2	30 3				
	SD	+ 1 35	+ 3 16	+ 2 15	± 11 87	12 9	42 0	30 8	17 5
	No	154	154	149	158				
5—9	Mean	11 5	38 2	29 8	30 1	13 5	43 8	30 8	13 3
	SD	1 53	± 3 80	± 2 56	± 11 14				
	No	1 6	156	151	159				
10—14	Mean	12 4	40 2	30 9	24 9	13 9	44 8	31 1	12 7
	SD	1 18	+ 3 02	± 2 63	± 12 79				
	No	94	96	94	95				
Ad males	Mean	13 8	46 0	29 9	15 7	15 7	51 1	30 9	7 5
	SD	1 63	+ 3 93	+ 2 27	± 10 47				
	No	56	56	56	56				
Ad females (non pregnant)	Mean	12 6	42 2	29 8	23 0	14 2	46 0	30 9	16 0
	SD	1 48	+ 3 31	± 2 00	± 11 80				
	No	67	67	67	67				

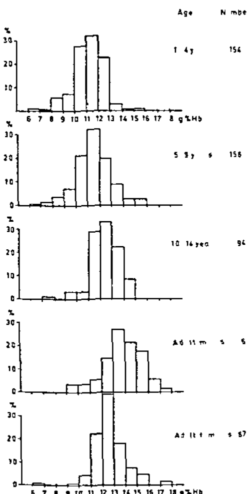


Fig 11 Relative distribution of Hb in children and adults in Ijaji

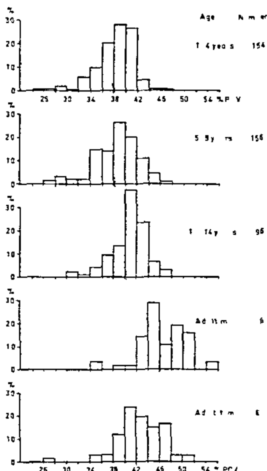


Fig 12 Relative distribution of PCV in children and adults in Ijaji

Anemia evaluation

As was discussed previously evaluation of anemia rates is a difficult task in the absence of fixed limits below which anemia can be said to exist As this study was part of a public health applied nutrition program in a developing country based in important aspects on the views of WHO it was decided to use the criteria of WHO with regard to hemoglobin levels below which anemia could be said to exist (see Chapter III) These WHO values were adjusted by +5 % for altitude (actual WHO value × 1 05) Because of the special hematological conditions during the first few months of life the children below 6 months will not be considered here

In Table 19 the percentages of anemia according to these criteria are presented. It is obvious that even without any altitude adjustment the anemia rate was very high in all age groups and highest during the second six months of life

When altitude adjusted anemia limits given

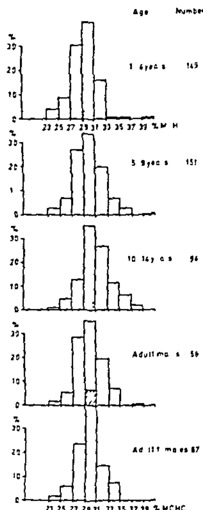


Fig 13 Relative distribution of MCHC in children and adults in *Ijaji*

Table 19 *Percentage of anemia in different age groups in Ijaji according to criteria of WHO See text*

	WHO anemia limits	
	not adjusted for altitude	adjusted by + 5 %
6/12—11/12	65 0	80 0
1— 4 years	32 5	48 1
5— 9 years	49 4	68 6
10—14 years	50 0	69 2
Ad males	26 8	44 6
Ad females	29 9	53 7
Total	47 6	59 0

non pregnant

by Natvig *et al* (82, 83) were applied (see Chapter III), the following percentages could be classified as anemic 5—9 years 55 8 % 10—14 years 35 1 %, adult males 67 9 % and adult females 70 2 %

Boys had a slightly higher rate of anemia than girls in the ages 1—14 years The difference was greatest in the group 5—9 years (77 2 for boys and 59 7 % for girls) For 1—4 years these figures were 49 3 and 46 8 % and for 10—14 years 70 6 and 65 4 %, respectively The sex distribution in the 6—11 month age group was very uneven (7 boys, 13 girls) and in this group girls were more often anemic than boys (84 6 *vs* 71 4 %)

In the following 3 main reasons for this high prevalence of anemia will be discussed anemia due to infections, anemia due to hookworm and nutritional anemia

Anemia and infection

During the clinical examination of the children in Ijaji in November 1965 it became evident that there was an exceedingly high frequency of infections of different kinds In Table 20 some of the common types in children 0—14 years are recorded The figures refer to the blood tested children

A history of gastroenteritis and upper respiratory infection refers to some time during the 2 weeks preceding the examination and is defined for gastroenteritis as 4 or more watery and/or mucous and/or bloody stools per day with or without vomiting and for upper respiratory tract infection as 3—4 of the following symptoms fever difficulties in breathing sneezing and coughing

Skin infection refers to bacterial infections such as *tropical ulcer pyodermia impetigo and the like*

Chiggers is an intracutaneously or subcutaneously located infestation with the sand flea usually under the toes or toe nails causing a local chronic infection most often with lymph gland enlargement in the groin

As can be seen in Table 20 the prevalence of gastroenteritis and upper respiratory infections was high already during the infant year and continued to be high for enteritis

during the pre school age after which a decrease was noted The scabies rate increased with increasing age during childhood as also did chiggers while the skin infection rate was about 30 % after 1 year These impressive figures illustrate well the poor hygienic standard prevailing in such a rural village in spite of the fact that water is available all the year round

The adults underwent an abbreviated form of clinical examination and the results are not fully comparable with those of the children However in all clinically examined adults the rate of scabies was only 5 1 % and skin infection 1 8 % while axillary and or inguinal lymph gland enlargement was diagnosed in 21 8 % Some skin affections may



Fig 15 Scabies with secondary infection on the hands



Fig 14 Scabies with secondary infection on the feet and lower legs



Fig 16 Impetigo

Table 20 *Symptoms and signs of infections in blood tested children in Ijaji and Backo in November and December 1965 See text for explanation*

Age	Enteritis	Upper resp inf	Scabies	Skin inf	Chiggers	Other inf	Lgl enlarged	No infection
<1 year (33)	27 3	24 [illegible]	27 3	12 1	0	15 1	3 0	42 4
1—4 years (161)	36 6	14 3	43 5	[illegible]8 9	36 6	36 7	34 [illegible]	1[illegible] 4
5—9 years (163)	10 4	4 9	44 8	30 7	63 8	5 1	5[illegible] 8	4 3
10—14 years (97)	3 1	7 2	52 6	27 8	56 7	[illegible]6 8	51 5	16 [illegible]

Fungus inf scalp
Otitis
Signs of lung inf
Relapsing fever
Fever of unknown origin (in a few cases more than one of these infections appeared in the same child)

have been missed as the subjects could not be examined naked Chiggers was not recorded although it was not infrequently seen as was also the case with a variety of scratches and fissures on the feet

The high mean values for ESR (Table 18) should be regarded in the light of these clinical findings Already during the first half year of life mean ESR was nearly 30 mm and throughout childhood it remained high The lowest mean ESR was recorded in adult men 25—34 years of age (11 9 mm), while in the non pregnant females there was a tendency to increasing values with increasing age (17 6 mm in the 15—24 year group compared with 29 2 mm in females ≥45 years)

In Table 21 the percentages of subjects with ESR ≤10 mm and with ESR ≤20 mm are given It will be seen that the great majority in all age groups exceeded 10 mm In ages 1—9 years only a small fraction had ESR ≤10 mm The majority, except in adult males also exceeded 20 mm

In Fig 17 the subjects are divided according to the level of their ESR and the mean Hb is calculated for each ESR group In all age groups there was a decreasing mean hemoglobin value with increasing sedimentation

Table 21 *Percentage of subjects with low (≤10 mm) and 'acceptable" (≤20 mm) ESR in Ijap*

Age group	ESR ≤10 mm	ESR ≤20 mm
<1 year	12 1	39 4
1— 4 years	1 9	18 4
5— 9 years	3 1	20 1
10—14 years	13 7	37 9
Ad males	35 7	75 0
Ad females	13 4	47 8
Total	9 5	32 4

rate The differences between the highest and lowest ESR groups amounted to 1 4—2 9 g % after 1 year and are highly significant It should be noted, however, that from 10 years and upwards there were but few subjects in the highest ESR groups The difference in mean Hb between the lowest and intermediate ESR groups was highly significant for adult males and almost significant for children 5—9 years and adult females, while in the remainder *the difference was not significant*

For comparison, the mean Hb in healthy subjects in Addis Ababa is also given As will be seen there was more than 1 g % difference in mean Hb between those with ESR ≤20 mm

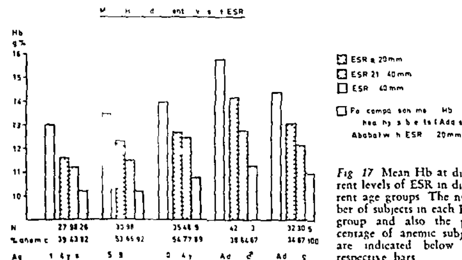


Fig 17 Mean Hb at different levels of ESR in different age groups The number of subjects in each ESR group and also the percentage of anemic subjects are indicated below the respective bars

in Addis Ababa and Ijaji in all age groups although the altitude could only account for 0 2—0 3 g %

In Fig 17 it is also seen that the percentage of anemic subjects in all age groups was higher in the higher ESR groups and *vice versa* It should however be noted that although this trend was obvious a considerable proportion of subjects with ESR ≦20 mm were anemic

Anemia and hookworm

The mean Hb values in different age groups in subjects with positive and negative micro scopic hookworm diagnoses can be seen in Fig 18 In all age groups the mean Hb value in subjects with a positive diagnosis was lower than in subjects with a negative stool test The difference about 1 g % in ages 1—14 years but smaller in the adults is highly significant in the 5—9 year group significant in the 1—4 year group almost significant in the 10—14 year group and not significant in the adults It is also evident from Fig 18 that as a consequence the percentage of anemic subjects was higher in the hookworm groups It should be noted however that a considerable percentage in the non hookworm groups were also anemic Two infants were diagnosed as having hookworm (20 samples examined) They were 8 and 11 months old with Hb values of 10 3 and 8 2 g % respectively

The material in some age groups is rather small It should also be emphasized that the direct microscopy method does not reveal the extent of the infestation as does the egg counting method

Nutritional anemia
a Iron deficiency

The anemic subjects were divided into those with MCHC below 27 % and those >27 % This limit was chosen somewhat arbitrarily It corresponds approximately to the mean minus 2 SD for all age groups in the material of healthy and apparently healthy subjects in Addis Ababa (Chapter III) In the non anemic subjects in Ijaji none hade an MCHC value below 27 % In the anemic subjects on the other hand a value below 27 % was recorded in a high percentage in the smaller children (see Fig 19 in which also the *total* anemia rates in the different age groups are recorded) The pre school age group was divided into children 12—35 months old and those 36—59 months The rate was highest 43 8 % during the second half year of life and decreased gradually during the pre-school years From 3 years and upwards it ranged between 7 7 and 16 0 %

In other words the anemia was found to be of a clearly hypochromic type in a much higher percentage from 6 months to 3 years than after 3 years

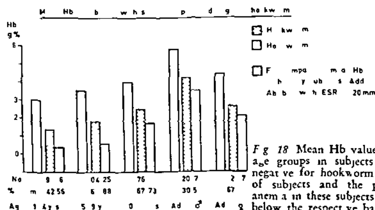


Fig 18 Mean Hb values in different age groups in subjects positive and negative for hookworm The number of subjects and the percentage of anemia in these subjects are indicated below the respective bars.

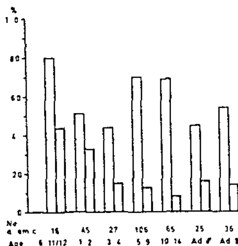


Fig 19 Percentage of anemic subjects in different age groups (open bars) and percentage of anemic subjects with MCHC below 27 % (shaded bars)

In subjects with a positive hookworm diagnosis 37 5 % 13 6 and 12 5 % of the anemic cases in the age groups 1—4, 5—9 and 10—14 years, respectively had MCHC values below 27 % No adults with hookworm and anemia had MCHC below 27 % However, rather few adults gave a stool sample In no case did non anemic subjects with hookworm have an MCHC value below 27 %

Therapy trial During the course of the applied nutrition program, children with frank anemia were given oral iron medication repeatedly Experiences have shown, however that in practically all cases the treatment has been interrupted after only a few days or a week due to lack of interest or understanding

In November 1967, a therapy trial with injectable iron (Jectofer®) was started and all children and adults with frank anemia were called for injections (intramuscular), which were planned to continue for about 1 week The total dose aimed at raising the Hb to 10—12 g % 6 weeks later the blood was to be checked again Unfortunately it was possible to persuade only 5 out of 40 (4 adults and 1 child) to attend and have their blood re checked, giving an indication of the problems involved in running seemingly un sophisticated programs

As can be seen in Table 22, four of the 5 subjects improved Three had a very low initial MCHC, 22—23 %, indicating iron deficiency anemia One pregnant woman, although responding by improvement, was still considerably anemic at the end of the trial (Hb 8 4 g %) probably due to a too small dose of iron

Serum iron and transferrin were determined in 3 cases A 9-year old child displayed the typical picture of iron deficiency (SI 30 µg % and TIBC 396 µg %, saturation 7 6 %), *improving after treatment* (SI 77 µg % TIBC 364 µg % and saturation 21 2 %) The second case (no 140 in Table 22), not responding with raised Hb to iron treatment, had SI and TIBC within mean minus 1 SD for apparently healthy females in Addis Ababa on both occasions (SI 80—83 µg %, TIBC 269—283 µg %)

Table 22 *Result of therapy trial with Jectofer® intramuscularly in 5 subjects in Ijaji*

No	Sex	Age	Before Hb g %	Before MCHC	After Hb g %	After MCHC	Hookworm
2571	M	9	6 6	22 0	10 3	27 8	pos
204	F	26	6 8	22 7	8 4	26 3	—
115	F	41	9 9	28 3	12 1	31 0	pos
156	M	55	10 8	23 0	12 4	29 5	neg
140	F	60	10 8	29 2	10 9	30 3	pos

pregnant

b Folic acid and vitamin B 12

In 17 females (3 pregnant) and 1 male determination of folic acid and vitamin B 12 was made 9 of them were anemic and 9 non anemic In all cases the results were within normal limits (in this laboratory the normal limits for vitamin B 12 are 151—900 pg/ml and for folic acid 3 1—15 ng/ml)

c Protein calorie malnutrition

In the assessment of the nutritional status in community surveys of this type the weight/age ratio has been recommended (65) as the most simple method giving a satisfactory indication of the prevalence of protein calorie malnutrition

Although it is important to keep in mind that there are multiple factors involved in producing a poor weight development an attempt was made to correlate the weight/age ratio to hemoglobin values in children aged 0—14 years An indication of the general weight development is given in Fig 20 As there is yet no local reference standard the Harvard standard was used for the ages 0—59 months and thereafter the Iowa standard (see Jelliffe (65) for references) For each child the weight/age was expressed as per cent of this standard as suggested by Jelliffe (65) and the means calculated The standards differ slightly causing a jump after 5 years

During the first half year the weight/age ratio was close to the American standard (97 %) Although the number of children in this group was small 11 the finding was consistent with the widely recognized fact that weight development is satisfactory during the first 4—6 months in developing countries the time when breast milk alone is adequate food and the rate of infection still fairly low After 6 months due to inadequate food and a stream of infections the physical development starts to lag behind The mean weight/age during the second half year in the Ijaji children was down from 97 % to 80 % and remained at about this level throughout childhood

In Fig 25 the mean Hb value for each weight age group has been calculated for the different age groups and also the percentage of anemia in each of these groups

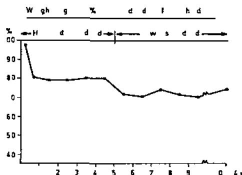


Fig 20 Mean weight/age ratio expressed as per cent of Harvard and Iowa standards in Ijaji children See text



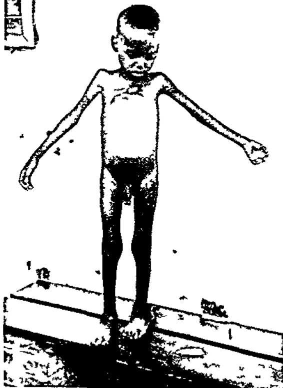


Fig 21 Less than 70 % weight/age



F g 23 Moderate prote n malnutr t on Note muscular hypoton a



F g M d a p ote n calo e malnutr t on Note swollen mph ands n the gro ns caused by h gers



F g 24 More than 90 % we ght a_e

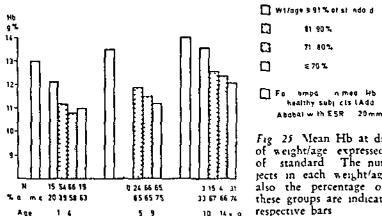


Fig 25 Mean Hb at different levels of weight/age expressed as per cent of standard The number of subjects in each weight/age group and also the percentage of anemia in these groups are indicated below the respective bars

In the group less than 1 year the number of children in each weight/age group was small Most children aged less than 6 months were found in the weight/age group ≥91 % and as has been shown earlier a higher mean Hb value was noted at this age as compared to the second half year of life Infants are therefore excluded from this table

In the age group 1—4 years the differences in mean Hb between the highest and lowest weight/age group was highly significant In the group 5—9 years there was no child at all in the weight/age group ≥ 91 % The difference in mean Hb between the next best weight/age group and that ≦70 % was not significant In the 10—14 year age group finally, the difference was almost significant There were, however only 3 children in the best group

It should also be noted that with decreasing mean Hb the percentage of anemic subjects in the lower weight/age groups consequently increased

The gap in mean Hb from the healthy children in Addis Ababa should be noted although the 3 children of 10—14 years in the best weight/age group were equal to their Addis Ababa mates when the difference in altitude was taken into consideration

Non infected children

As was discussed previously a great number of the children presented symptoms and signs of infections of various kinds and the ESR was found to be increased in the majority of cases It is assumed that many of these infec tions especially when combined or when severe or chronic cause a decrease in hemoglobin concentration or a slight to moderate anemia (17) In the following an attempt is made to study separately those children who on clinical examination showed no symptoms or signs of infection

As is indicated in Table 20 this was a comparatively small percentage It should however, be pointed out that children with conjunctivitis (acute or chronic as well as trachoma cases), with nasal discharge and with gum affections have not been excluded but are to be found among the non infected children Children with slight fungal affection of the scalp are also included Bilateral lymph gland enlargement on the neck and in the axillae and groins were classified in the clinical examinations as + (slight) for pea-bean size, + + (moderate) for bean-plum size, and + + + (severe) for more than plum size Any palpable enlargement of more than pea size at least in the axillae and groins

should be considered pathological and at least under the prevailing conditions should indicate the presence of or recently passed infection in the respective drainage area Children with a total lymph gland score of ++ or more were arbitrarily included in the infected group

In the vast majority of cases the children had combinations of various infectious diseases such as a history of diarrhoea and chiggers with lymph gland enlargement.

Not a single child would have been classified as non infected if the signs mentioned above such as conjunctivitis and running nose had also been included.

As the adults underwent a different, abbreviated form of examination not fully comparable with that used for children and as it was not possible to examine them undressed only children will be discussed here

Children 0—11 months 14 of 33 children were [illegible]sed as "non infected" (42 4 %) The ESR [illegible] as however above 20 mm in 8 [illegible]es (range 22—38 mm)

[illegible] were below 6 months of age [illegible] was anemic (Hb 9 5 g %) This [illegible] months old and had a weight/age [illegible] % but an ESR raised to 35 mm, [illegible] a passed or undiagnosed [illegible]

[illegible] were 6—11 months old. If the WHO [illegible] adjusted for altitude was app[illegible] Hb [illegible] than 11 3 g %) 6 of them [illegible] with Hb ranging between 6 [illegible] g % The child with Hb 6 3 g % [illegible]onths old and had a weight/age ra[illegible] and signs of wasted muscles He wa[illegible] only breast milk and cow's milk (like [illegible] of the others in this group) and had a c[illegible] on deficiency anemia (MCHC 21 [illegible] %) A[illegible] the non infected children in this age group had an MCHC below the mean for healthy children in Addis Ababa although only 2 had below 27 % 3 had rickets

Children 1—4 years 20 of 161 children were classified as non infected (12 4 %) The ESR was above 20 mm in 13 cases (21—45 mm) 7 were classified as anemic and 10 non anemic The Hb value ranged from 8 4—15 3 g % In 3 cases Hb was not recorded The mean weight/age was about 80 % with a range of 67—101 % In no fewer than 8 cases clinical signs of malnutrition (thin or wasted muscles dyscolored hair) were recorded and one child was in a state of pre kwashiorkor — an 18 months old boy with Hb 10 8 g % and ESR 4 mm The other 6 cases of anemia could be attributed to hookworm (1 case) dietary iron deficiency (milk only 2—3 cases) and subclinical infections as indicated by raised ESR (32—42 mm)

4 of the 5 children for whom Hb was recorded and with ESR ≤20 mm were not anemic The 5th was the child referred to above with pre kwashiorkor

Children 5—9 years 7 of 163 children were classified as non infected (4 3 %) The ESR was above 20 mm in 6 cases (range 25—39 mm) 4 children were anemic and 3 non anemic (Hb range 8 8—14 3 g %)

The weight age ratio was lower than in the younger children probably due to the use of a different standard and ranged from 61—79 % However none showed any clinical hair or muscle signs of malnutrition and none showed a positive stool test for hookworm (5 tested) although one anemic child with Hb 8 8 g % and MCHC 24 1 % was positive in 2 subsequent stool tests With the exception of this child none was severely anemic.

Children 10—14 years 16 of 97 children were classified as 'non-infected (16 5 %) 7 of 15 had an ESR above 20 mm, with a range of 22—50 mm (*sic'*) In one case ESR was not recorded 9 children were anemic and 6 non-anemic (no Hb record in one case) The Hb ranged between 10 8 and 14 8 g %

The weight/age ratio varied considerably (48—97 %), probably due in part to unprecise age information One child had dyscolored hair but no other signs of protein malnutrition and 2 had hookworm one of whom showed signs of iron deficiency anemia (Hb 10 8 g % MCHC 24 5 %)

Of the 7 children with ESR ≦20 mm, 3 were anemic — one was the child referred to above with hookworm, the other 2 were marginally low

Children with weight/age ratio above 90 %

In the age group 1—4 years the mean weight/age ratio was stable at about 80 % of the Harvard standard (see Fig 20) The age determination can be considered satisfactory as the vast majority were examined several times after the base line examination and each time an attempt was made to arrive at an even more exact age utilizing a local calendar Before 1 year nearly all children dropped successively in weight/age ratio and after 5 years the age determination started to be more difficult For these reasons only the children of ages 1—4 years with a weight/age ratio of more than 90 % of the standard were selected for consideration Only 15 out of 154 (9 7 %) blood-tested children then remained (see also Fig 25) Their weight/age ratio ranged between 91 and 116 %

12 were non anemic (Hb 11 3—15 3 g %) and 3 anemic (Hb 8 7—11 1 g %) Even if these absolute figures are small, the rate is considerably lower than in the total group (20 and 48 % respectively) The anemia could be ascribed to hookworm in 2 cases while in the third child there was no stool record All 3 however, also had signs of infections according to previously discussed criteria wasted muscles and moderate liver enlargement were noted in 2 of the anemic children

Of the 12 non anemic children only 4 had no infection , although in 3 of these 4 the ESR was above 20 mm (21—28 mm) The infected non-anemic cases had ESR values between 15 and 45 mm

Serum iron and transferrin

The result of SI and TIBC determinations in children 5—14 years (55 boys and 21 girls) and in adults are given in Table 23

Table 23 *Results of determinations of Hb, ESR, SI, TIBC and per cent transferrin saturation in children and adults in Ijaji*

Age group		Hb	ESR	SI	TIBC	% satur
5—14 years	Mean	12 6	25 4	65	314	20 8
	S.D	± 1 02	± 9 95	±28	± 46	± 8 5
	No	76	75	76	76	76
Adult males	Mean	13 6	17 2	78	282	29 0
	S.D	± 1 66	±10 7	±33	± 51	±12 1
	No	38	38	53	53	53
Adult females	Mean	12 1	25 8	68	288	24 0
	S.D	± 1.54	±11 9	±32	± 43	±11 1
	No	33	33	53	53	53

refers to subjects where SI and TIBC were determined.

The mean value for SI in children was markedly below that of healthy children in the same age group in Addis Ababa (65 and 93 µg %, respectively, a highly significant difference) while the mean TIBC values agreed well

Adult males showed an even greater and highly significant difference in mean SI from healthy and apparently healthy subjects in Addis Ababa, (78 and 125 µg % respectively) with nearly the same mean TIBC

The difference in SI for adult females was smaller (68 and 94 µg %, respectively) although also highly significant

52 7 % of the boys and 52 4 % of the girls, or a total of 40 were anemic The mean value for SI in the anemic children was 53 µg % and that in the non-anemic group 78 µg % The difference is highly significant The mean value for TIBC was similar in both groups — 313 µg % in the anemic group and 316 µg % in the non-anemic group

None had severe anemia and none displayed the typical picture of severe iron deficiency anemia with marked hypochromia (MCHC below 27 %) low SI and high TIBC

Of the adults, there was no significant difference in mean SI in males between anemic ($n = 15$) and non anemic ($n = 23$) subjects (77 and 79 µg %), while in the females there was a significant difference (57 and 83 µg % $n = 24$ and 9, respectively) The mean values for TIBC were very similar for all the four groups, in anemic and non anemic males these values were 279 and 280 µg % and in females 283 and 295 µg %

The high mean ESR should be noted ESR above 20 mm was recorded in 72 0 % in the children in 28 9 % in the adult males and in 69 7 % in adult females In children as well as adults, the mean SI was higher in the groups with ESR ≤20 mm, although the differences were not significant (in children 74 and 62 µg %, in adult males 82 and 68 µg % and in adult females 77 and 59 µg %)

There was no significant difference between SI in subjects with and without hookworm In children with hookworm ($n = 15$) the mean SI was 64 µg % and in those without ($n = 31$) it was 61 µg % In adults (males and females) with hookworm this value was 61 µg % ($n = 9$) and in those with a negative stool result 81 µg % ($n = 43$)

Pregnant females

At the clinical examination all females were asked whether they were pregnant Some females in the early stages of pregnancy may have concealed their condition or not been aware of it and may thus have been included in the non pregnant group

It was not possible to obtain any exact gestational age in the 14 pregnant females but all were most certainly in the second and third trimester In 10 of them Hb and PCV determinations were made Only 1 of these was anemic (Hb 6 8 g %, MCHC 22 7 %) She responded by a moderate improvement after iron injections (see above) but probably did not get adequate amounts of iron for a sufficiently long period Her serum folic acid and vitamin B 12 values were within the normal range No stool was given for hookworm examination

The others ranged between 11 6 and 13 7 g % with a mean value of 12 6 ± 0 87 g % which in fact is the same as for the non pregnant females in Ijaji The mean value, including the anemic woman, was 12 0 g %

The mean SI was 98 ± 33 µg % ($n = 11$) — the anemic woman had no SI or TIBC determinations Although the material is small it should be noted that the mean SI was well above that of both males and non pregnant females in Ijaji and was at the same level as in non pregnant apparently healthy females in Addis Ababa (94 µg %)

Comments

It was considered useful and necessary to present this material in some detail to give an indication of the complexity of the situation Compared with Western standards very few of the subjects in such a community are healthy in the strict sense The causative factors are multiple and not easy to differentiate when evaluating the role of each factor in, for instance, the etiology of anemia

In the following, however, an attempt will be made to discuss briefly some of the more important factors contributing to the high degree of the usually mild to moderate anemia found in these investigations

It was not possible in the field to make red blood cell counts and the only index available was MCHC Garby *et al* (32) have stressed that this index is an insensitive method for diagnosing mild form of iron deficiency anemia The reported normal value for MCHC varies a great deal, probably depending on the techniques used (32, 79 109, 123) but it has the advantage of not being influenced by altitude (57) It may seem inappropriate to select only those anemic cases who had an MCHC below the mean minus 2 SD in the normal material' in Addis Ababa, for the diagnosis of hypochromic iron deficiency anemia However in view of the satisfactory iron depots found in the bone marrow hemosiderin study it must be reasonable to assume that this criteria would give a rough idea about how much *iron deficiency contributes to the overall percentage* of anemia It has been pointed out that this type of anemia is the predominant one in the tropics (62, 88), forming a formidable public health problem The index MCHC has been used in this study, utilizing the normal material in Addis Ababa as a reference standard and with a liberal range (mean — 2 SD), thereby possibly underestimating the frequency of iron deficiency anemias

The anemia of infection is usually normochromic but may be hypochromic although the hypochromia is not as marked as in iron deficiency anemia (17)

If the criteria for iron deficiency anemia mentioned are used it would be resonable to assume that a conservative estimation of this type of anemia could be made The overall rate was 16 9 % — or roughly one sixth of all anemias would be of the iron deficiency type

There was, however, a general tendency to hypochromia in Ijaji compared to the healthy material in Addis Ababa (Table 18), most marked in the small children The overall rate of marked hypochromia (MCHC <27 %) in subjects more than 1 year of age was 9 1 % compared to 3 3 % in Addis Ababa This should be matched by the rate of MCHC >35 0 % or mean + 2 SD for Addis Ababa This figure was 2 6 % in Ijaji and 2 3 % in Addis Ababa (cf Figs 13 and 19) In other words, marked hypochromia was about 3 times more common in Ijaji

The fact that the highest rate was found among small children (Fig 19) agrees with the food pattern, providing a low dietary iron intake during the first 1½ —2 years when the diet consists mainly of milk and starchy gruels (101) After this age the children start to participate in the adult (iron rich) meals and the hypochromic anemia seen in older children and adults is presumably caused to a larger extent by iron drain rather than low intake It would be reasonable to assume also that the high frequency of diarrhoeal diseases, *striking hardest the infant and pre school* groups (Table 20) would also contribute to an impaired iron absorption during this period of limited intake and rapid growth

No chemical studies have been performed regarding the intake of folic acid (and vitamin B 12), although green leafy vegetables for instance kale is consumed and also, though not too frequently, meat (101) The fact that none of the 18 subjects examined had abnormal serum folic acid and B 12 levels does not exclude the existence of megaloblastic anemia This type of anemia is more common in fe-

males and especially in pregnant females The fact that 17 of the tested subjects were females, 9 of whom were anemic and 3 pregnant, one with a severe anemia, may indicate that if folic acid or B 12 dependent anemias exist they may not constitute a major problem Further studies in this field are desirable, however

The role of protein-calorie malnutrition *per se* in the etiology of anemia is very difficult to evaluate In Rhesus monkeys fed a low protein diet (105) a normoblastic, normochromic anemia developed, as well as a marked reduction in the serum iron, transferrin, albumin and the total serum proteins An interesting point was that the gamma globulins showed an increase All these changes could be reversed by re feeding, although the gamma globulins remained high During the time of the low protein diet the animals also had a reduced iron absorption The anemia was moderate in contrast to the severe reduction in albumin and the marked atrophy of organs with a high protein turnover indicating that hemoglobin synthesis was maintained at the expense of other body proteins

A reduced erythroid activity in the bone marrow in animal studies (105) as well as in patients with severe protein calorie malnutrition (34 103) has been postulated as the cause, resulting in a decreased cellular proliferation and interpreted (103) as an expression of adaptation to undernutrition However the anemia of kwashiorkor may have a multiple etiology or the etiology may be dependent on a locally prevailing deficiency pattern (28 34 90) With protein feeding associated deficiencies may become apparent or more marked (34)

In the present study the children did show a low weight/age ratio and also a low weight/height ratio and in about 1/3 of them there were clinical signs of protein malnutrition mainly thin or wasted muscles and thin or dyscolored hair but only occasionally was frank kwashiorkor or marasmus seen (56)

The weight/age ratio in the blood tested privileged children in Addis Ababa was in the pre school group slightly above 90 % and after that about 85 % thus about 10 % higher than in Ijaji but still not 'optimal', although none showed any clinical signs of protein malnutrition

The significant differences in mean Hb between the highest and the lowest weight/age groups (1—4 and 10—14 years Fig 25) do not necessarily indicate a relationship between protein malnutrition *per se* and low hemoglobin concentration but may merely indicate that a number of factors which cause a poor weight development, such as chronic or frequently recurring infections, at the same time cause a decreased Hb or frank anemia The fact that the children 1—4 years with a weight/age ratio of >90 % had less anemia but still had infections in a high percentage (although less than the whole group) does not necessarily speak against this

It must be admitted that although there may be some indications that protein deficiency *per se* may play a part in contributing to the generally low hemoglobin levels in Ijaji, its exact role cannot be established

A total of 17 9 % of the blood tested subjects ≥1 year for whom stool samples were examined were positive for hookworm (Fig 18) In all ages above 1 year the overall rate of anemia in subjects with hookworm was 73 % and in those with no hookworm 55 % The difference would indicate the percentage of anemia due to hookworm infestation (68) This would be 18 %, which is about 1/4 of all anemias seen in the hookworm positive group Apparently there remains 55 % anemia to be accounted for

There were significant differences between mean Hb values in hookworm positive and negative children 1—14 years old but not in adults The MCHC value was below 27 % in a high percentage (37 5 %) in hookworm positive anemic cases of ages 1—4 years In older children this percentage was lower and in adults with hookworm none had a value

below 27 % This indicates that the small children seem to be more vulnerable to the blood loss caused by the worm, than older children and adults

Some caution may be necessary in interpreting the figures given Generally hookworm infestation was more common in the pure farming areas in the outskirts of the village than in the center although positive cases were seen from all areas The health condition was less good among the farmers and in particular the rate of infections among their children was considerably higher It cannot be excluded, therefore, that factors other than hookworm contribute substantially to the higher rate of anemia found among the hookworm positive subjects and that the statement that 1/4 of all anemias found in this group are due to hookworm is an overestimation However, hookworm seemed to be responsible to a larger extent for the severe anemias A total of 26 subjects in all age groups had Hb values below 9 g % In 15 of these stool samples were examined and 9, or 60 % were positive for hookworm which is about 3 times the overall rate

In comparison with more heavily infested areas in the world (29, 31, 68, 107) the prevalence of hookworm in the Ijaji area is moderate and the degree of anemia encountered must be considered modest Therapy trials with small amounts of iron in areas heavily infested with hookworm without deworming (29 31), have resulted in improved hemoglobin values although the response was even better when antihelminthics were given simultaneoulsy (31) It would therefore seem reasonable to conclude that the high dietary iron intake in this community protects from the development of the more serious and widespread iron deficiency anemias

There are reasons to believe that chronic or recurring acute infections is the major cause of the anemia diagnosed in this study It is well known that a mild non-progressive anemia usually normochromic, not seldom hypochromic, develops as a result of chronic infections and a variety of other chronic disorders (17, 123) Tables 20 and 21 illustrate well the infection situation and it is probable that the situation is even more serious than what can be revealed in a survey examination which by necessity must be rather superficial For instance cases of primary tuberculosis and a variety of other dis orders requiring a more refined set up of diagnostic aids may have been missed This is also supported by the high number of raised ESR in non-infected children Although the level of ESR does not necessarily completely reflect the state and severity of infection it is widely used and accepted as such an indicator The relationship between a high ESR and low hemoglobin concentration in this study seems clear (Fig 17) and cannot possibly be explained by the slightly raised ESR which can be caused by anemia *per se* (123)

The generally low levels of serum iron in Ijaji compared to the healthy subjects in Addis Ababa should also be regarded in relation to the high frequency of infections and high levels of ESR The mean SI was lower in children and adult males and females with ESR above 20 mm although the difference was not significant An interesting point is that the mean TIBC values were very similar to those in Addis Ababa TIBC falls during infections although the reduction is proportionally smaller than that of serum iron (40), and also in states of plasma protein depletion such as in kwashiorkor and nephrosis (8) There was however, no clinical evidence of marked protein malnutrition in these subjects

All blood tested subjects were negative for malaria Very few cases were diagnosed in the village in 1965—1967, and here the disease was probably brought in from the lowlands it is possible that the disease will be more prevalent over the next few years with an increasing influx of people from malarial regions

As far as is known sickle cell anemia has not been diagnosed in central Ethiopia No investigations have yet been performed in the Ijaji area but this field of hematology deserves penetration

Summary

A comprehensive investigation of children was made in the village of Ijaji in the fall 1965, later supplemented with children from a nearby village and with adults from Ijaji, all examined during the same season A separate study on SI and TIBC was, however made in May (1967)

A total of 726 subjects in ages ranging from 1 month to more than 70 years underwent clinical and hematological examination

In general the Hb and PCV values were markedly below those of the healthy subjects in corresponding ages in Addis Ababa and overall the rate of anemia according to current WHO criteria was approximately 60 %

ESR was markedly increased in the large majority of cases and the mean values in different ages were well above those in the healthy subjects in Addis Ababa This was found to correspond to a high rate of infection, especially in children, and in a strict sense no child was uninfected

SI was significantly lower than in Addis Ababa while TIBC was found to be at the same level

Serum folic acid and vitamin B 12 determined in a limited number of cases, were within normal limits

About 18 % of the blood tested subjects above 1 year of age for whom stool samples were examined had hookworm Other helminths, although not reported here, were very frequently diagnosed, mainly ascaris

There was a generally low weight for age ratio in children after 6 months of age In the age group 1—14 years nearly 30 % were 70 % or less of the 'standard'

The anemia was clearly hypochromic (MCHC below 27 %) in a much higher percentage between 6 months and 3 years than after 3 years — 36 and 12 %, respectively — supporting dietary data, indicating a low iron intake until the children start to participate in the adult, iron rich food The hypochromic anemia diagnosed in older children and adults was probably due to iron drain rather than a low dietary iron intake

Reported evidence indicates that hookworm infestation struck hardest in the preschool age group in which the rate of hypochromic anemia in hookworm positive children was highest Overall about or less than 1/4 of all anemias in hookworm positive subjects seemed to be due to hookworm

The role of protein calorie malnutrition in the etiology of the anemia diagnosed is very difficult to evaluate for reasons discussed Although the mean Hb decreased with decreasing weight for age ratio other factors may have been the primary cause of this

In view of the evidence presented it is concluded that the main causative factor for the generally low hemoglobin values or mild to moderate anemia and the low serum iron values is infections of various kinds Combinations of different etiological factors are, however, most probably in operation in a major proportion of the subjects studied.

General Summary

Ethiopia may represent the country with the highest daily intake of dietary iron in the world This is true at least in the highlands where the grain tef is the staple food It was therefore considered of interest 1 to study the sources of this iron, 2 to investigate certain hematological values in healthy and apparently healthy subjects of different ages consuming a diet with this high iron content (for adults in the order of 300—500 mg per day) 3 to study the iron stores of different age groups and — for comparison — 4 to investigate certain hematological parameters and the frequency and type of anemia in a representative Ethiopian highland village, in which the iron intake is equally high

As it was suspected that a substantial part of the iron consumed with the tef was derived from the iron rich soil, 8 tef samples which had first been cleaned in the traditional way, were further cleaned by washing 20 times in diluted hydrochloric acid Generally the iron content was thus reduced to between $^1/_8$ and $^1/_4$ of the values obtained after the traditional cleaning, indicating that most of the iron is to be found *on* the tef as contamination but that the iron content *in* the tef would still be 3—4 times that in wheat This would mean a daily iron intake from the tef grain itself for an adult male of approximately 75—100 mg If contributions from other food items are included (in the order of 25 mg) the total daily iron intake from the food itself will far exceed recommended allowances and the actual intake in most other countries

In Chapter III the results of hematological examinations of 615 healthy and apparently healthy subjects of ages 1—65 years are presented 78 of these were pregnant females In addition 30 newborn children had capillary blood tests and another 47 had cord blood examined

In general the mean hemoglobin values in the different age groups agreed well with accepted standard values after an altitude adjustment of the latter by +7 % Also the mean values for SI and TIBC agreed satisfactorily with those of other workers In pregnant women and delivering mothers there was only a comparatively small decrease in mean Hb values during the last trimester compared to non-pregnant females and no decrease at all in SI while TIBC was raised as is usual in pregnancy The anemia rate in subjects 1—65 years of age was low, approximately 5—7 % In adults no certain cases of iron deficiency anemia were diagnosed with the criteria used, while the figure in children was about 3 %

In Chapter IV the result of an investigation of bone marrow hemosiderin in 136 healthy and apparently healthy subjects of ages 3—65 years indicated an increase of bone marrow iron with increasing age In subjects 30 years or more +++ — ++++ hemosiderin (scale 0 — ++++), was diagnosed in about 80 % All except 3 young children had stainable hemosiderin In 19 cases with bone marrow hemosiderin of trace — ++++, a desferrioxamine test (500 mg given intramuscularly) was made and 6 hours iron excretion in the urine determined Although the iron excretion was higher in the group with hemosiderin +++ — ++++ compared to the group trace — ++, the difference was not significant and there was a considerable scattering of individual values The highest excretion value was 1 43 mg For reasons discussed, the findings are interpreted as indicating that in the subjects who underwent bone marrow studies the increased iron was mainly localized reticuloendothelially and that this would represent an early and comparatively mild iron overload state This is supported by chemical iron analysis of 98 autopsy liver specimens, 76 of which were from subjects who died of trauma (6 females), in which the mean non hemin iron

values were not significantly raised above those of hematologically normal Swedish males Although in this material there was a high incidence of suicide and homicide, subjects probably in a less satisfactory nutritional condition, the results indicate nevertheless an adequate or more than adequate iron nutrition

In Chapter V the results of hematological investigations of 726 subjects, of ages 1 month to more than 70 years, in a representative Ethiopian highland village are presented No less than 60 % were diagnosed as having anemia 17 % of which was of the iron deficiency type The prevalence of iron deficiency anemia was much higher in the ages 6 months to 3 years than after 3 years, when the dietary iron intake is high About 18 % of the blood-tested subjects above 1 year of age for whom a stool sample was examined had hookworm, but probably less than 1/4 of the anemia cases diagnosed in this group were due to hookworm *per se* In 18 cases the serum folic acid and vitamin B 12 values were examined and found within normal limits There was, especially among children, a very high prevalence of infections of various kinds and the majority of the subjects studied had raised ESR Although protein-calorie malnutrition may have played a part in the etiology of anemia, it is concluded that the main causative factor is repeated acute or chronic infections

References

1 Ågren G *et al* To be published

2 Albritton E C (Edit) Standard values in blood W B Saunders Comp Philadelphia 1952

3 Allen D M Dean R F A The anemia of kwashiorkor in Uganda Trans roy Soc trop Med Hyg 59 326 1965

4 Almgård G High content of iron in teff Eragrostis abyssinica Link and some other crop species from Ethiopia — a result of contamination LantbrHogsk Ann 29 215 1963

5 Areskog N H Selinus R Vahlquist B Physical work capacity in relation to nutritional status To be published

6 Biedermann P Fitzgerald J D Keberle H Studies in the development of a screening test of iron storage disease Acta hemat 33 297 1965

7 Bothwell T H Bradlaw B A Siderosis in the Bantu A combined histopathological and chemical study Arch Path 70 279 1960

8 Bothwell T H Finch C A Iron metabolism J and A Churchill Ltd London 1962

9 Bothwell T H Finch C A Iron losses in man In Occurrence causes and prevention of nutritional anaemias Symposia of the Swedish Nutrition Foundation VI (Ed G Blix) 1967

10 Bothwell T H Isaacson C Siderosis in the Bantu A comparison of incidence in males and females Brit med J I 522 1962

11 Bothwell T H Pirzio Biroli G Finch C A Iron absorption I Factors influencing absorption J Lab clin Med 51 24 1958

12 Bothwell T H., Seftel H Jacobs P., Torrance J D Baumslag N Iron overload in Bantu subjects Studies on the availability of iron in Bantu beer Am J clin Nutr 14 47 1964

13 Bratteby L E Studies on erythro-kinetics in infancy XI Acta paediat scand 57 215 1968

14 *Brise H Hallberg L Iron absorption studies II* Absorbability of different iron compounds Acta med scand Suppl 376 23 1962

15 Brise H Hallberg L Iron absorption studies II Effect of ascorbic acid on iron absorption Acta med scand Suppl 376 51 1962

16 Brown A E The health situation and problems in Ethiopia 1966 — a paper designed to assist in formulation of the third five year plan Unpublished paper April 1966

17 Cartwright G E The anemia of chronic disorders Seminars in hematology 3 351 1966

18 Cartwright G E Wintrobe M M The anemia of infection XVII A review Advanc intern Med 5 165 1952

19 Charlton R W Bothwell T H Hemochromatosis dietary and genetic aspects In Progress in hematology (Ed E B Brown C V Moore) Grune and Stratton New York 1966

20 De Silva C C Fernando R P Anemias of Ceylonese children Israel J med Sci 2 499 1965

21 Edington G M Hemosiderosis and anemia in the Gold Coast African W Afr med J 3 66 1954

22 Edington G M Nutritional siderosis in Ghana Cent Afr J Med 5 186 1959

23 Elwood P C Utilization of food iron — an epidemiologist's view Nutr et Dieta 8 210 1966

24 Fielder H J Reissig H Lehrbuch der Bodenkunde Vischer Verlag Jena 1964

25 Fielding J Differential ferrioxamine test for measuring chelatable body iron J clin Path 18 88 1965

26 Fielding J O'Shaughnessy M C Brunström G M Differential ferrioxamine test in idiopathic haemochromatosis and transfusional haemosiderosis J clin Path 19 159 1966

27 Food composition tables — minerals and vitamins — for international use FAO Nutritional studies No 11 1954

28 Foy H Kondi A Macdougall L Pure red cell aplasia in marasmus and kwashiorkor treated with riboflavin Brit med J I 937 1961

29 Foy H Nelson G S Helminths in the etiology of anemia in the tropics with special reference to hookworms and schistosomes Exp Parasit 14 240 1963

30 Gale E Torrance J Bothwell T H The quantitative estimation of total iron stores in bone marrow J clin Invest 42 1076 1963

31 Gandra, Y R Bradfield R B Hernandez, V *Dias C Firbas H J Studies on the pathogenesis* of a tropical normocytic anemia Am J clin N r 18 116 1966

32 Garby L Killander A Definition of anemia In Occurrence causes and prevention of nutritional anaemias Symposia of the Swedish Nutrition Foundation VI (Ed G Blix) 1967

33 Gerritsen T Walker A R P The effect of habitually high iron intake on certain blood values in pregnant Bantu women J clin Invest 33 23 1954

34 Ghitis J Velez H Linares F Sinisterra L., Vitale J J Cali Harvard Nutrition project II The erythroid atrophy of kwashiorkor and marasmus Am J clin Nutr 12 445 1963

35 Gilles H M Williams E J W Ball P A J Hookworm infection and anemia Quart J Med 33 1 1964
36 Gillman J Gillman T Structure of the liver in pellagra Arch Path 40 239 1945
37 Gillman T Hathorn M Canham P A S Experimental dietary siderosis Am J Path 35 349 1959
38 Gillman T Hathorn M Lamont N M Liver disease in Durban Africans Histopathological findings in biopsies S Afr J med Sci 23 187 1958
39 Gillman J Mandelstam J Gillman T A comparison of chemical and histological estimations of the iron and copper content of the livers of Africans in relation to the pathogenesis of cytosiderosis and cirrhosis (haemochromatosis) S Afr J med Sci 10 109 1945
40 Hagberg B Studies on the plasma transport of iron Acta paediat Suppl 93 1953
41 Hagberg B Lundstrom P Intravenous iron in normal pregnancy Acta obstetr gyn scand 34 212 1955
42 Hahn P F Carothers E L Darby W J Martin M Sheppard C W Cannon R O Beam A S Densen P M Petersen J C McClellan G S Iron metabolism in human pregnancy as studied with the radioactive isotope Fe^{59} Am J obstet gynec 61 477 1951
43 Hallberg L Hedenberg L The effect of Desferrioxamine on iron metabolism in man I Scand J Haematol 2 67 1965
44 Hallberg L Hedenberg L Desferrioxamine induced urinary iron excretion in normal and iron deficient subjects Scand J Haematol 4 11 1967
45 Hallberg L Hedenberg L Weinfeld A Liver iron and desferrioxamine induced urinary iron excretion Scand J Haematol 3 85 1966
46 *Hallgren B Hemoglobin formation and storage* iron in protein deficiency Acta soc Med upsaliensis 49 79 1953
47 Handbook of biological data (Ed W S Spector) W B Saunders Comp Philadelphia and London 1956
48 Hansen H A Weinfeld A Hemosiderin estimations and sideroblast counts in the differential diagnosis of iron deficiency and other anemias Acta med scand 165 333 1959
49 Harker L A Funk D D Finch C A Evaluation of storage iron by chelates Am J Med 45 105 1968
50 Hathorn M Gillman T Canham P A S Lamont N M Plasma iron and iron binding capacity in African males with siderosis Clin Sci 19 35 1960
51 Hegsted D M Finch C A Kinney T D The influence of diet on iron absorption II The interrelation of iron and phosphorus J exper Med 90 147 1949
52 Hemoglobinopathies and allied disorders Report of a WHO scientific Group Wld Hlth Org techn Rep Ser 338 1966
53 Higginson J Gerritsen T Walker A R P Siderosis in the Bantu of Southern Africa Am J Path 29 779 1953
54 Higginson J Grobbelaar B G Walker A R P Hepatic fibrosis and cirrhosis in man in relation to malnutrition Am J Path 33 29 1957
55 Hiyeda K The cause of Kaschin — Beck s disease Japanese J med Sci 4 91, 1939
56 Hofvander Y Eksmyr R To be published
57 Hurtado A Merino C Delgado E Influence of anoxemia on the hemopoietic activity Arch intern m d 75 284 1945
58 Hussain R Walker R B Layrisse M Clark P Finch C A Nutritive value of food iron Am J clin Nutr 16 464 1965
59 Hytten F E Leitch I The physiology of human pregnancy Blackwell Oxford 1964
60 ICNND Bolivia nutrition survey A report by the Interdepartmental Committee on Nutrition for National Defense June 1964
61 ICNND Ethiopia nutrition survey A report by the Interdepartmental Committee on Nutri*tion for National Defense September 1959*
62 Iron deficiency anemia Report of a study group Wld Hlth Org techn Rep Ser 182 1959
63 Isacson C Seftel H C Keeley, K J Bothwell T H Siderosis in the Bantu Relationship between iron overload and cirrhosis J Lab clin med 58 845 1961
64 Jacobsson K Personal communication
65 Jelliffe D B The assessment of the nutritional status of the community WHO Geneva 1966
66 Johansson S G O Mellbin T Vahlquist B Immunoglobulin levels in Ethiopian preschool children with special reference to high concentration of immunoglobulin E (IgND) Lancet I 1118 1968
67 Laurell C B Studies on the transportation and metabolism of iron in the body Acta physiol scand Suppl 46 1947
68 Layrisse M Roche M The relationship between anemia and hookworm infection Am J Hyg 79 279 1964
69 Lee K S The influence of environmental factors on micro sedimentation rate Eth med J 5 221 1967

70 Losowsky M S Effect of desferr oxam ne in pat ents w th ron load ng w th a s mple method for est mat n ur nary ron J cl n Path 19 165 1966
71 Mac Donald K A Hemochromatos s and he mos deros s C. C. Thomas Spr ngf eld Ill no s USA 1964
72 Mac Donald R A Pr mary hemochromatos s nher ted or acqu red? In Progress n hematology (Ed E B Brown C V Moore) Grune and Stratton New York 1966
73 Mac Donald R A Pechet G S T ssue ron and hemochromatos s Comparat ve geograph c stud es n Ireland Israel Japan South Africa and the Un ted States Arch nt Med 116 381 1965
74 Maley R W The effect of room temperature on erythrocyte sedimentat on rate and ts correc t on J cl n Path 10 354 1957
75 Mayet F G H Bothwell T H Hepat c ron stores in three d fferent rac al groups S Afr J med S i 29 55 1964
76 Melak H Mengesha Chem cal compos t on of teff (Eragrost s tef) compared w th that of wheat barley and gra n sorghum Econom c Botany 20 268 1966
77 Mellb n T *et al* To be publ shed
78 M nn ch V Okçuoglu A Tarkon Y Arcasov A C n S Yorukglu O Renda F Dem rag B P ca n Turkey II Effect of clay upon ron absorpt on Am J cl n Nutr 21 78 1968
79 Moe P J Normal red blood p cture dur ng the f r t three years of l fe Acta paed at scand 54 69 1965
80 Moore C. V Iron nutr t on In Iron meta bol sm An nternat onal symposium (Ed F Gross) Springer Verlag Germany 1964
81 Moore C. V The absorpt on of ron from foods In O currence causes and prevent on of nutr t onal anaem as Sympos a of the Swed sh *Nutr t on Foundat on VI (Ed G Bl x) 1967*
82 Natv g H Vellar O D Stud es on hemoglob n values n Norway VIII Hemoglob n hema tocr t and MCHC values in adult men and women Acta med scand 182 193 1967
83 Natv g H Vellar O D Andersen J Stud es on hemoglob n values n Norway VII Hemoglob n hematocr t and MCHC values among boys and g rls aged 7—20 years n elementary and grammar schools Acta med scand 182 183 1967
84 N lsson L Menstruat onsforluster och jarnbr st In Jarnbr st och jarnterap Nord kt symposium Goteborg 1964 (Ed L Hallberg)
85 Oh W L nd J Venous and cap llary hema tocr t n newborn nfants and placental trans fu on Acta paed at scand 55 38 1966
86 Ok n J T Treger A. Overy H R. Weil J V Grover R F Hematolog c response to med um h gh alt tude Rocky Mtn med J 63 44 1966
87 Panchurst R An h stor al exam nat on of trad t onal Eth op an med cine and surgery Eth med J 3 157 1965
88 Patwardan V N Nutr tional anem as n the Trop cs East Afr med J 39 200 1962
89 Patwardan V N Nutr t onal anem as — WHO research program Ea ly developments and progress report of collaborat ve stud es Am J cl n Nutr 19 63 1966
90 Per era S M Baker S J Hematolog al stud es n kwa h orkor Am J cl n Nutr 18 413 1966
91 P rz o-B rol C Bothwell T H F nch C A Iron absorpt on II The ab orpt on of rad o ron adm n stered w th a standard meal n man J Lab cl n Med 51 37 1958
92 Postmus S A report on a survey of the nutr t onal needs of ch ldren in Eth op a FAO 58 5 3339
93 Rath C E F nch C. A Sternal marrow hemos der n A method for the determ nat on of ava lable ron stores n man J Lab cl n Med 33 81 1948
94 Recommended D etary Allowances 6 h rev sed ed t on 1964 Nat onal Academy of Sc ences Nat onal Research Counc l Publ cat on 1146
95 Reynafarje C Lozano R Vald v eso J The polycythem a of h gh alt tudes ron metabol sm and related aspects Blood 14 433 1959
96 Ro en B J Tull s J L S mpl f ed defer roxam ne test n normal d abe c and ron-over load pat ents J Amer med Ass 195 261 1966
97 Pybo G Jarnbrist och grav d tet In Jarnbr st och arnterap Nord skt sympos um Goteborg 1964 (Ed L Hallberg)
98 S hade A L Oyama J Re nhardt R W M ller J R. *Bound ron and unsaturated ron* b nd ng capac ty of serum rap d and rel able quant tat ve de erm nat on Proc Soc exp B ol med 87 443 1954
99 Scr mshaw N S Taylor C E Gordon J E Interact on of nutr t on and infect on Am J med Sc 237 367 1959
100 Seftel H C Keeley K J Isaacson C., Both well T H S deros s in the Bantu The cl n cal nc dence of hemachromatos s in d abetic subjects J Lab clin Med 58 837 1967
101 Sel nus R *et al* To be publ shed
102 Shah d N T D amond L K., Shwachman H Anem a assoc ated w th prote n def c ency A study of 2 cases with cyst c f bros s. J Ped at. 59 533 1961

103 Smith N J Nutrition and anemia Israel J med Sci 2 405 1966

104 Sood S K Banerji L Ramalingaswami V Occurrence of nutritional anaemias in tropical countries. In Occurrence causes and prevention of nutritional anaemias Symposia of the Swedish Nutrition Foundation VI (Ed G Blix) 1967

105 Sood S K Deo M G Ramalingaswami V Anemia in experimental protein deficiency in the Rhesus monkey with special reference to iron metabolism Blood 26 421 1965

106 Spruyt D J, Eldor F B Messing S D Wade M K Ryder B Prince J S Yohannes T Demonstration and evaluation project Ethiopian health center program Eth med J 5 conference suppl 1967

107 Stott G Anaemia in Mauritius Bull Wld Hlth Org 23 781 1960

108 Strom J Mikromethode fur die Senkungsreaktion Acta pediat 14 567 1933

109 Sturgeon P Studies of iron requirements in infants III Influence of supplemental iron during normal pregnancy on mother and infant A The mother Brit J Haemat 5 31 1959

110 Sturgeon P Studies of iron requirements in infants III Influence of supplemental iron during normal pregnancy on mother and infant B The infant Brit J Haemat 5 45 1959

111 Tayback M M Prince J S Infant mortality and fertility in five towns of Ethiopia Eth med J 4 11 1965

112 Treger A, Shaw D B, Grover R F Secondary polycythemia in adolescents at high altitude J Lab clin Med 66 304 1965

113 Trowell H C Non infective disease in Africa Edward Arnold Ltd London, 1960

114 Turnberg L A Excessive oral iron therapy causing hemochromatosis Brit med J 1 1360 1965

115 Vahlquist B Das Serumeisen Eine padiatrisch klinische und experimentelle Studie Acta pediat Suppl 5 1941

116 Vahlquist B Normalvarden for hamoglobin roda blodkroppar och index uttryckta i absoluta tal for olika åldrar Svenska Lakartidn 45 2471 1948

117 Vahlquist B Children s Nutrition Unit — an Ethio-Swedish project in the field of health J Amer med Wom Ass 21 753 1966

118 Wainwright, J Siderosis in the African S Afr J Lab clin Med 3 1, 1957

119 Walker A P P Correction of haematological data for altitude Letter to Editor Trans roy Soc trop Med Hyg 50 509 1956

120 Walker A R P Arvidsson, U B Iron overload in the South African Bantu Trans roy Soc trop Med Hyg 47 536 1953

121 Weinfeld A Storage iron in man Acta med scand Suppl 427 1964

122 Weinfeld A Lundin P, Lundvall O Significance for the diagnosis of iron overload of histochemical and chemical iron in the liver of control subjects J clin Path. 21 35 1968

123 Wintrobe M M Clinical hematology 6th edition Lea & Febiger Philadelphia 1967

Acta Medica Scandinavica

Supplementum 495

Circulatory Adaptation at Rest and During Exercise in Normal Adults

A statistical evaluation of differences according to sex, age and body build

by Gustav Schroder

Distributed by Almqvist & Wiksell, Stockholm Sweden

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv*, was founded in 1869 by Professor Axel Key, MD. In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*.

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway, Sweden and the Netherlands. Short preliminary reports (not exceeding two pages) are published promptly. The papers are published in English, French or German. Acta Medica Scandinavica is published on a non profit basis.

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each a year) include free supplements to the current volumes. During the past few years an average number of 17 supplements has been issued per annum.

Subscription Rates

Per annum = two volumes
In Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Sw. kr. 120, incl. postage
Other countries Sw. kr. 140, incl. postage

Chief Editor

Associate Professor Birger Strandell, MD
Birger Jarlsgatan 9
S 111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Biblioteksgatan 11
S-111 46 Stockholm, Sweden

Subscription and Distribution

Almqvist & Wiksell
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1, Sweden

Printers

Almqvist & Wiksells Boktryckeri AB
Box 616
S-751 25 Uppsala 1, Sweden

From the department of medicine I (professor L. Werkö) Sahlgrenska Hospital University
of Göteborg Göteborg Sweden

CIRCULATORY ADAPTATION AT REST AND DURING EXERCISE IN NORMAL ADULTS

A statistical evaluation of differences according to sex, age and body build

BY

GUSTAV SCHRÖDER

GÖTEBORG 1968

ORSTADIUS BOKTRYCKERI AKTIEBOLAG
GÖTEBORG 196
14006

Contents

Introduction

Hemodynamic studies have become a part of the routine examination of patients with circulatory disorders of any kind An interpretation of these demands thorough information on normal circulatory data both at rest and during exercise

Many of the previously performed studies in man have, however, been confined to one sex or certain age groups The results are therefore of limited value in the clinical evaluation of circulatory disorders Various recording techniques have also been used It will thus not be proper to combine the results of different authors using different techniques in order to evaluate the influence of age sex and body build on cardiac performance in different body positions and degrees of physical exercise The pooling of different populations also introduces additional uncertainty The populations may change with time and location

The present series of investigations intend to elucidate the reaction of some circulatory and respiratory variables in circulatory normal adults of both sexes, utilizing a single established technique The cardiac output and the intraarterial blood pressure have been recorded simultaneously with collection of expired air at rest in the recumbent och sitting position and during exercise sitting on a bicycle ergometer

Special attention was given to influence of age, body size and sex on the variables, heart rate, arterial pressures cardiac output, pulmonary ventilation oxygen consumption and the various variables calculated from combination of these Sex differences of the variables were analysed before and after adjustment for differences in age and body build

I Studies at rest in the recumbent position

There is a considerable number of reports on the systemic hemodynamic findings in resting recumbent subjects (for references see Wade & Bishop (53) Smulyan *et al* (45)) The influences of age, body size and sex have also been studied The influence of the combination of these factors is less known The influence of age and body surface area have been investigated by Brandfonbrener *et al* (9) and Jegier *et al* (30) The relationship between body surface area and cardiac output have been studied more recently by Smulyan *et al* (45) An approach using simple linear and multiple regressions to study various relationships of the hemodynamic reaction to age and body size has been made by Strandell *et al* (48)

Subjects

All subjects had a history free from cardiovascular disease A physical examination and an ECG at rest were also free from abnormalities The upper limit for blood pressure by the Korotkoff method was set at 150/90 mm Hg

Forty-four subjects 24 men and 20 women were included in the present study The age range for men was 18—59 and for women 18—56 years Statistics regarding age and anthropometric data are given in table I and fig 1

Eleven men and 14 women were ambulatory paid volunteers from various social and occupational groups The other subjects were inpatients who were considered to be 'normal' with respect to the circulatory system

Eighteen men were engaged in predominantly manual work and six in sedentary work Two were students Thirteen men were inpatients, 10 of whom were studied during convalescence from peptic ulcer disease Fifteen *women were engaged in mainly manual* work, 13 of these were house-wives Another three women had different types of light office work One was a student Five of the six hospitalized women were admitted for mammaplasty *

*) The admitting diagnosis, age height weight occupation as well as the individual measurements are available in tables which can be obtained from the author upon request

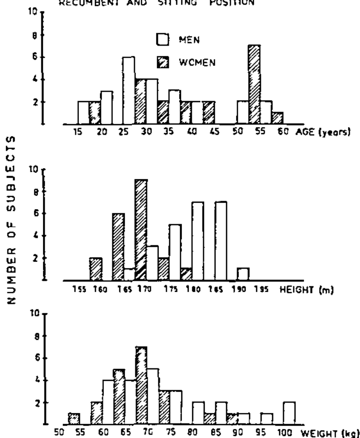


Fig 1 Histogram for distributions of age height and weight

Table I *Anthropometric data and age in 24 men and 20 women studied in recumbency Mean values ($\bar{x}$) standard deviations (s_x)*

		MEN		WOMEN	
		$\bar{x}$	s_x	$\bar{x}$	s_x
Age	(years)	33.5	11.7	39.2	12.5
Height	(m)	1.811	0.0 8	1.657	0.049
Weight	(kg)	77.3	16.1	67.1	8.2
Body surface area	(m)	1.965	0.190	1.739	0.118

Performance

All subjects were investigated in the morning. They had fasted for 12 hours. Premedication was not given. The in-patients were studied after an average hospital period of six days (range 1—17 days).

The subjects were informed about the procedure in advance. Care was taken to avoid apprehension during the procedure. The study was made in a quiet room by a doctor, a nurse and an assistant.

The brachial artery was punctured percutaneously at the antecubital fossa using local anesthesia. A polyethylene catheter, No. 205, 40 cm long, was threaded 10—20 cm proximally over a metal guide wire which was introduced through the needle as described by Seldinger (43). The catheter was flushed every 5—10 minutes with a few ml of physiological saline to which had been added heparin (25 mg/1000 ml).

An antecubital vein was catheterized by the same technique. The tip of a radioopaque thin walled Ödman-Ledin catheter (KIFA AB, Sweden) was placed into the right atrium or superior vena cava under fluoroscopy in 32 subjects and in the other 12 subjects a polyethylene catheter No. 160 was similarly threaded in the central direction 10—30 cm but without fluoroscopic guidance.

A slow drip of the flush solution was started for the venous catheter.

After a resting period of about 30 minutes, the recordings of heart rate, intraarterial blood pressure and cardiac output were started simultaneously with the expired air collection.

Complications of importance were seen only once. One woman, age 53 years, experienced ischemic pains after the puncture of her left forearm, the radial pulse of which became unpalpable. The circulation improved within a couple of months but one year later she still had paresthesias of the hand probably due to injury of the median nerve.

Methods

Heart rate. An ECG from the precordial leads was monitored continuously on an oscilloscope. The ECG was also recorded by mean of a five channel photographic oscillograph (Klinik Type EM 130, Elema Schonander AB).

Arterial blood pressures. The catheter was connected to a pressure transducer of variable inductance type (EMT 490 A, Elema-Schonander AB) feeding an amplifier (EMT 455, Elema-Schonander AB). The pressure curves were recorded by the oscillograph. The amplitudes of pressure were linear to pressures in the ranges used. The zero level for pressure was placed 5 cm below the sternal notch.

The systolic, diastolic and mean pressures were estimated by the hand-smoothing method of the curves from at least

10 beats The mean arterial pressure was obtained by electrical integration

The pressures at rest were recorded immediately before and after every cardiac output determination The average values were used

Hematocrit Arterial blood was collected in Ellerman tubes After mixing a column of 50 mm was taken up into two capillary tubes The capillaries were centrifuged for 15 minutes at 6000 r p m (approximately 3600 g) Average values of the height of the packed blood cells to total column in per cent are reported No correction was made for trapped plasma

Cardiac output A modified dye-dilution technique, based on the original principle of Stewart (47) and Hamilton *et al* (21 22) with bromsulphalein as the indicator was used Bromsulphalein yields values for the cardiac output that are in close agreement with those obtained by the Fick method or with other dye indicators as Evans Blue and Cardiogreen (16 36, 56)

The dye was injected rapidly through the venous catheter At the same time a rotating drum with Ellerman tubes was started to collect arterial blood in one or two seconds samples each of about 2 ml The dye remaining in the catheter was slowly redrawn into the syringe starting about 5 seconds after the injection After centrifugation the extinction values of bromsulphalein in the plasma were read at a wave length of 580 mμ in a Beckman B spectrophotometer A curve of the extinction values was plotted on semilogarithmic paper The extinction values of descending slope were extrapolated The cardiac output in l/min (Q) was calculated according to the formula

$$Q = k \cdot \frac{E_i}{E_a} \cdot \frac{100}{100\text{-Hct}}$$

Amount of dye injection (E_i), the sum of the extinction values from the extrapolated curve (E_a), hematocrit (Hct) and a time constant (k)

Systemic vascular resistance The quotient, mean arterial blood pressure in mm Hg over cardiac output in l/min was used to express the total systemic vascular resistance Right atrial pressure was not used as a correction

Systemic vascular conductance The inverted value of the resistance multiplied by 1000 was used

Stroke volume The mean heart rate from an ECG recorded during the cardiac output procedure was used to calculate the stroke volume which was expressed in ml

Left ventricular work Left ventricular work in kpm per min was estimated as cardiac output times mean arterial pressure times 13 6/1 000 Left atrial pressure was not included as a correction

Left ventricular stroke work Left ventricular work was divided by heart rate and the stroke work was expressed in pm (pondmeter)

Oxygen consumption The subjects breathed through a short rubber mouthpiece into a low-resistance valve system and a volume of about 25—50 l of expired air was collected in a Douglas bag The volume was measured by means of gas flow-meter (Elge 2,

Langebelsenkirchen) The total ventilation was expressed in liters per minute, at body temperature, pressure saturated with water vapor (BTPS) Samples of expired air were analysed in duplicate for oxygen and carbon dioxide according to Scholander (42) and the oxygen consumption in ml per minute at standard temperature pressure dry (0° C, 760 mm Hg) (STPD) was calculated

Arteriovenous oxygen difference The Fick formula was used for calculating the arteriovenous oxygen difference in ml per liter from the quotient oxygen consumption over cardiac output

Body surface area The method of DuBois and DuBois (15) was used to estimate the body surface area The weight to the nearest 0 1 kg was used The height was measured to the nearest cm

Statistics

Mean values and standard deviations were calculated according to current formulas (10) Linear regression equations according to method of least squares were calculated using the age, height, weight and body surface area as independent variables (10) At first simple linear regressions were calculated When the same dependent variable had more than one regression on the independent variables a trivariate regression was calculated (37) A fixed combination with age and body surface area and another with age, weight and height as independent variables was also calculated for each dependent variable as significant dependences can be lost One independent variable not significant in simple linear regression can be significant in combination with another independent variable For a survey of multiple regression technique see Moore (37)

Curvilinear regressions were not calculated as the plots did not indicate curvilinearity of the regressions

Simple regressions with slopes being significant at least at the 5 per cent level were considered Some multiple regressions with slopes of one of the independent variables only on the indicative level (0 10 > P > 0 05) were also considered The two tailed t tables were used for analysis of the coefficients of regression Adjusted mean values and residual standard deviations were calculated from the significant regression equations (10) The mean values of the independent variable of the other sex were used for adjustment

Sex differences of mean values and adjusted mean values were tested according to t-test when no differences of variances were evident according to F-test (46) When the variances were different a t-test was used with adjustment of the degree of freedom according to Welch (58)

Comparisons of two regression equations were made by the conventional covariance method (10)

When correlation of significance was

obtained between the independent variables the method of partial correlation was used to get the pure influence of each independent variable (10).

Calculations of the statistics for skewness (g_1) and kurtosis (g_2) were made to check the normality of distribution. A t-test was performed to detect the significant deviations of g_1 and g_2 (46). Only deviations at least at the 5 % level of g_1 and g_2 are reported.

A desk top automatic computer (Programma 101, Olivetti) was used for most calculations. Programs were written for all the calculations. For more complicated multiple regression calculations, a greater automatic computer (FACIT EDB 3) programmed with standard library programs and fed by taped data was used.

Many calculations were also made by use of mechanical desk computers.

Results

Mean values, standard deviations and probability of differences are given in table II. Linear regression equations on age and anthropometric measurements are given in table III. Adjusted values to the mean values of anthropometric and age recordings of the other sex are given in table IV together with sex comparisons.

Table II. *Mean values ($\bar{x}$), standard deviations (s_x) and P values (P) of sex differences of hemodynamic and respiratory data in 24 men and 20 women. For abbreviations see text.*

		MEN		WOMEN		Sex differences of	
		$\bar{x}$	s_x	$\bar{x}$	s_x	Mean values P	Variances P
HR	beats/min	64.3	8.9	68.6	11.5	–	–
$P_{S_{BA}}$	mmHg	123.9	12.2	129.2	10.4	–	–
$P_{D_{BA}}$	mmHg	72.6	6.2	73.2	6.1	–	–
$\bar{P}_{BA}$	mmHg	89.6	8.0	94.1	7.9	–	–
$P_{S_{BA}}-P_{D_{BA}}$	mmHg	51.3	8.1	56.0	6.7	<0.05	–
Q	l/min	6.83	1.22	5.69	0.87	<0.005	–
SV	ml	107.7	23.3	83.8	11.1	<0.001	<0.01
LVW	kpm/min	8.28	1.79	7.27	1.27	<0.05	–
LVSW	gm	130	28	107	19	<0.001	<0.05
SVR	mmHg l/min	13.51	2.59	16.96	3.36	<0.001	–
SVC	ml/min/mmHg	76.5	14.0	60.9	10.0	<0.001	–
$\dot{V}O_2$	ml/min (STPD)	285.6	33.1	220.8	22.4	<0.001	<0.05
$\dot{V}_E$	l/min (BTPS)	9.27	3.27	6.95	1.54	<0.005	<0.01
R		0.845	0.114	0.818	0.106	–	–
$(a-\bar{v})O_2$	ml/l	42.8	6.9	39.5	5.7	<0.1	–
Hct		41.7	2.9	38.4	3.1	<0.005	–

Table III *Linear regressions of hemodynamic and respiratory variables (y) on the independent variables (x) age (years) height (m) weight (kg) and body surface area (m^2) in 24 men (m) and 20 women (f) during rest in recumbency s_{yx} residual standard deviation b regression coefficient and s_b the standard deviation of regression coefficient b/s_b t value for regression coefficient r correlation coefficient*

Eq nr	y		x	Regression equation	s_{yx}	b/s_b	P	r
1	P_{SBA} P_{DBA}	m	Weight	y = 32 1+0 248 x	7 17	2 26	<0 02	0 494
2	P_{SBA}	f	Age	y = 112 4+0 428 x	9 16	2 53	<0 025	0 513
3	P_{DBA}	f	Weight	y = 48 5+0 368 x	5 42	2 41	<0 05	0 494
4	P_{DBA}	f	BSA	y = 32 7+23 3 x	5 55	2 15	<0 05	0 452
5	$\bar{P}_{BA}$	f	Age	y = 81 1+0 331 x	6 88	2 61	<0 02	0 523
6	Q	m	Height	y = − 11 48+10 11 x	1 097	2 83	<0 02	0 481
7	Q	m	BSA	y = 0 88+3 02 x	1 105	2 50	<0 025	0 470
8	Q	m	Age x_1 BSA x	y = +1 92−0 0372 x_1 +3 054 x_2	1 06	x_1 1 75 x 2 64	<0 10 <0 02	0 565
9	SV	m	Weight	y = 40 5+0 869 x	19 1	3 51	<0 005	0 597
10	SV	m	BSA	y = −39 3+74 8 x	18 9	3 62	<0 005	0 610
11	LVW	m	Weight	y = 4 71+0 0462 x	1 67	2 13	<0 05	0 414
12	LVW	m	BSA	y = 0 088+4 26 x	1 64	2 38	<0 05	0 451
13	LVW	f	Weight	y = 2 35+0 0733 x	1 16	2 26	<0 05	0 469
14	LVW	f	BSA	y = −2 10+5 39 x	1 13	2 43	<0 05	0 498
15	LVSW	m	Weight	y = 37 4+1 20 x	20 5	4 48	<0 001	0 691
16	LVSW	m	BSA	y = −65 7+99 5 x	20 8	4 38	<0 001	0 681
17	SVR	m	Height	y = 58 10−24 6 x	2 20	3 13	<0 005	−0 554
18	SVR	m	BSA	y = 25 0−5 84 x	2 39	2 23	<0 05	−0 429
19	SVR	m	Age x_1 BSA x_2	y = 22 69+0 0724 x_1 −5 908 x	2 28	x_1 1 79 x_2 2 36	<0 10 <0 05	0 540
20	SVR	f	Age x_1 BSA x	y = 34 16+0 1574 x_1 −13 44 x_2	3 03	x_1 2 40 x_2 1 94	<0 05 <0 10	0 523
21	SVC	m	Height	y = −152 9+126 6 x	12 2	2 86	<0 01	0 527
22	SVC	m	BSA	y = 15 1+31 2 x	13 0	2 20	<0 05	0 423
23	SVC	m	Age x_1 BSA x	y = 28 37−0 418 x_1 +31 62 x_2	12 27	x_1 1 92 x 2 35	<0 10 <0 05	0 550
24	SVC	f	Age x_1 BSA x	y = 7 59−0 481 x_1 +41 5 x	8 89	x_1 2 52 x 2 06	<0 025 <0 10	0 541
25	VO_2	m	Weight	y = 197 1+1 146 x	28 1	3 15	<0 005	0 556
26	VO_2	m	BSA	y = 98 0+95 5 x	28 3	3 08	<0 01	0 550
27	VO	f	Height	y = −221 4+266 8 x	18 8	3 00	<0 01	0 577
28	VO	f	Weight	y = 132 4+1 318 x	20 3	2 31	<0 05	0 479
29	VO	f	BSA	y = 37 4+105 5 x	19 2	2 82	<0 02	0 554
30	V_E	m	Height	y = −34 2+24 02 x	3 02	2 23	<0 05	0 428

Table IV *Adjusted mean values ($\bar{Y}|x$) and residual standard deviations (s_{yx}) for the mean values of age and body size of the other sex. Comparisons are made with the mean value of the other sex. If regression found for that sex the lowest residual standard deviation was used for testing. For abbreviations see the text*

Eq nr	Variable	Sex	Adjustment for	Adjusted values $\bar{Y}/x$	s_{yx}	Sex differences of Mean values P	Variances P
1	$P_{S_{BA}} - P_{D_{BA}}$	m	Weight	48.8	7.4	<0.005	—
2	$P_{S_{BA}}$	f	Age	126.8	9.44	—	—
3	$P_{D_{BA}}$	f	Weight	77.0	5.8	<0.025	—
4	$P_{D_{BA}}$	f	BSA	78.5	6.2	<0.005	—
5	$\bar{P}_{BA}$	f	Age	92.2	7.1	—	—
6	Q	m	Height	5.28	1.25	—	<0.05
7	Q	m	BSA	6.14	1.16	—	<0.05
8	Q	m	Age + BSA	6.35	1.12	<0.05	—
9	SV	m	Weight	98.8	19.7	<0.005	<0.01
10	SV	m	BSA	90.7	19.9	—	<0.01
11	LVW	m	Weight	7.81	1.72	—	<0.01
12	LVW	m	BSA	7.31	1.72	—	<0.01
13	LVW	f	Weight	8.01	1.23	—	—
14	LVW	f	BSA	8.49	1.26	—	—
15	LVSW	m	Weight	117.8	21.1	—	—
16	LVSW	m	BSA	107.4	21.8	—	—
17	SVR	m	Height	17.3	2.6	—	—
18	SVR	m	BSA	14.8	2.5	<0.02	—
19	SVR	m	Age + BSA	15.3	2.4	—	—
20	SVR	f	Age + BSA	13.0	3.5	—	<0.01
21	SVC	m	Height	57.1	14.2	—	<0.01
22	SVC	m	BSA	69.4	13.7	<0.025	<0.01
23	SVC	m	Age + BSA	66.9	13.0	—	<0.05
24	SVC	f	Age + BSA	73.1	10.3	—	—
25	VO_2	m	Weight	273.9	28.9	<0.001	<0.05
26	VO	m	BSA	263.9	29.7	<0.001	<0.05
27	VO	f	Height	261.6	23.6	<0.01	—
28	VO_2	f	Weight	234.2	21.5	<0.001	—
29	VO	f	BSA	244.7	21.4	<0.001	—
30	V_E	m	Height	5.59	3.49	—	<0.01

Heart rate (HR) Heart rate had no sex difference of mean values or variances and no significant regressions of heart rate on age or anthropometric data in either sex were found. The coefficient of skewness and kurtosis did not deviate significantly from normality in either sex.

Brachial arterial pressures Brachial arterial systolic ($P_{S_{BA}}$) diastolic ($P_{D_{BA}}$) mean ($\bar{P}_{BA}$) and pulse pressure ($P_{S_{BA}} - P_{D_{BA}}$)

The unadjusted mean value of pulse pressure for women was significantly higher than for men No other sex difference of mean values or variances was obtained for brachial arterial pressures

In men, there was a regression of pulse pressure on weight (1*) After weight adjustment, the sex difference was more pronounced

The women had a regression of systolic pressure on age (2*) The age adjusted value was not significantly different from the systolic pressure in men The diastolic pressure had no regression on age but one on weight (3) and one on body surface area (4) The weight and body surface area adjusted values were higher than the mean value of diastolic pressure in men The mean pressure of the women had a regression on age (5*) The age adjusted value was not different from the mean arterial pressure in men The pulse pressure of women had no significant regression

left ventricular work and stroke work as well as systemic vascular conductance were significantly higher and the systemic vascular resistance significantly lower in men when no adjustments were made for sex differences of age and body size

Stroke volume and left ventricular work had higher variances in men

In men regressions of cardiac output on each of height (6*), (fig 2) and body surface area (7*), (fig 3) were found Regression values of cardiac output for men adjusted to the mean value of height and body surface area of women were not significantly different from the mean value of cardiac output of women A smaller residual standard deviation was obtained when both age and body surface area were used as independent variables (8) The probability level of the dependence on age, however, was only indicative Adjustment for this trivariate regression did not eliminate the sex difference The women had no significant regressions for cardiac output

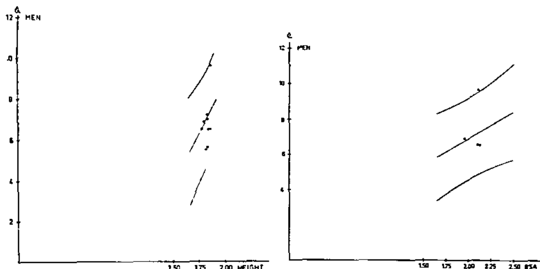


Fig 2 3 Regress on lines and 95 % confidence limits of the dependence on height (m) 2) and on body surface area (m) BSA 3) of cardiac output (l/min) Q in men

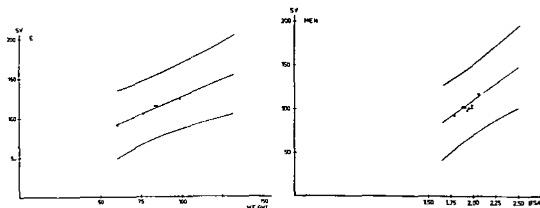


Fig 4 5 Regression lines and 95 % confidence limits of the dependence on weight (kg) 4) and body surface area (m) BSA 5) of the stroke volume (ml) SV in men

had regressions on body surface area for both sexes (12 14) Test of identity accepted the hypothesis of identical regressions Regressions on weight were also found (11 , 13) for which the hypothesis of identity was also accepted

The adjustments according to body size difference thus eliminated the sex difference of left ventricular work

The estimated left ventricular stroke work in men had a regression on each of weight (15) and body surface area

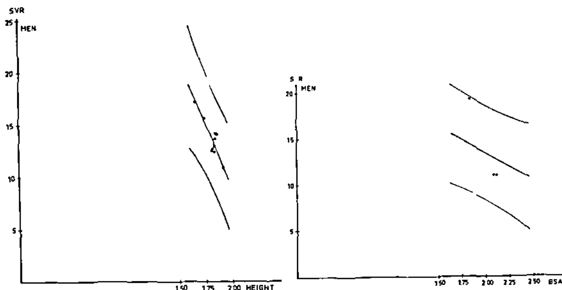


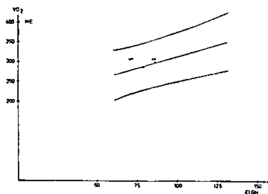
Fig 6 7 Regression lines and 95 % confidence limits of the dependence on height (m) 6) and on body surface area (m²) BSA 7) of the systemic vascular resistance (mmHg/l/min) SVR in men

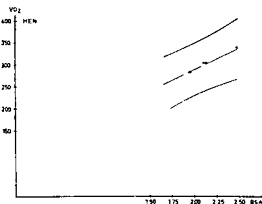
(16) When the stroke work of men was adjusted to each of the mean weight and body surface area of women no significant differences remained Women had no significant regressions

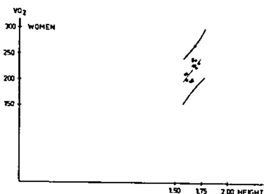
The systemic vascular resistance of men had regressions on height (17), (fig 6) and body surface area (18), (fig 7) The residual standard deviation for age and body surface area as independent variables was lower than for that of body surface area alone (19) The probability of dependence on age, however, was only indicative A dependence on age when combined with body surface area was obtained in the group of women (20˚) The dependence on body surface area, however, was only indicative The systemic vascular resistance of women had no single dependence on body size or age The adjusted value to the height of women was not different but the adjusted value to the body surface area of women was lower

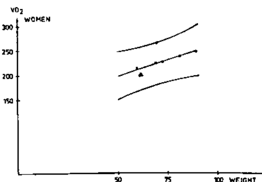
The systemic vascular conductance of men had regressions on each of height (21) and body surface area (22) The combined dependence on age and body surface area (23˚) had a lower residual standard deviation but the probability of dependence on age was only indicative Women had a regression on age when it was combined with body surface area (24) the dependence of which however, was only indicative On single anthropometric data or age as independent variable no regression was obtained Adjustment for height of the regression in men eliminated the sex difference but the adjustment for body surface area difference did not

In men deviations from normality of









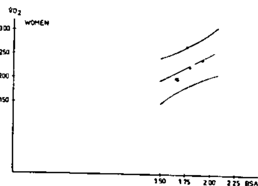


Fig 8 9 10 11 12 Regression lines and 95 % confidence limits of the dependence of oxygen consumption (ml min) VO) on weight (kg) 8) and on body surface area (m) BSA 9) in men and on height (m) 10) weight (kg) 11) and on body surface area (m) 12) in women

left ventricular work were found (g_1 = 2.24, P < 0.05 and g_2 = 2.66, P < 0.02) i.e. a long tail of high values and a peaked distribution.

Women had no deviation from normality of g_1 and g_2 for any flow dependent variable.

Respiratory variables. Oxygen consumption ($\dot{V}_{O_2}$), pulmonary ventilation ($\dot{V}_E$), ventilatory exchange ratio (R) and arteriovenous oxygen difference ((a—$\bar{v}$)$_{O_2}$).

The mean values of oxygen consumption and pulmonary ventilation were higher in men than in women. The variances of oxygen consumption and pulmonary ventilation were higher in men.

Men had regressions of oxygen consumption on each of weight (25*), (fig. 8) and body surface area (26*), (fig. 9). The adjusted values to the mean weight and body surface area of women did not eliminate the sex difference.

Women had regressions of oxygen consumption on each of height (27*) (fig. 10), weight (28*), (fig. 11) and body surface area (29*), (fig. 12). The adjusted values were significantly lower than the mean value for men. Test of identity of the regressions on each of weight and body surface area in the two sexes thus rejected that hypothesis (P < 0.001).

The pulmonary ventilation in men had a regression on height (30*). Adjustment to the height of the women eliminated the sex differences.

Women had no significant regressions for ventilation on body size or age.

Ventilatory exchange ratio and arteriovenous oxygen difference of men and women had no regressions on age or body size.

Hematocrit (Hct). Hematocrit was higher in men than in women. No significant regressions were found on age or body size in either sex.

The only deviation from normality of respiratory variables or hematocrit was found in men. Pulmonary ventilation had skewness as well as kurtosis (g_1 = 4.51, P < 0.001 and g_2 = 5.45, P < 0.001).

Discussion

The distributions of body size measurements in the present study are seen in fig. 1. Analysis of relationships between age and body size revealed no regression on age of the anthropometric data in men.

Women however had a regression for each of weight and body surface area on age. The regression equations were:

$y = 52.5 + 0.371 \cdot x$, (t (b/s_b) = 2.92, P < 0.01, r = 0.566, s_{yx} = 6.90) for weight (y) on age (x) and

$y = 1.545 + 0.00493 \cdot x$ (t (b/s_b) = 2.58, P < 0.02, r = 0.520 and s_{yx} 0.1034) for body surface area (y) on age (x).

Correlations between age and weight have also been reported in subjects of both sexes, whose arterial pressures were studied with respect to body size and age influence by Truedsson (51).

In men the coefficient of skewness and kurtosis for weight deviated significantly from normality ($g_1 = 1.64$, $P < 0.001$ and $g_2 = 3.16$, $P < 0.001$) that is a long tail of high values and a peaked distribution compared with a normal curve. Three subjects with the weights 130.8 kg, 100.5 kg and 98.5 kg contributed to these deviations.

Women had no deviation from normality of these coefficients for anthropometric data and age.

The deviations from normality are no serious drawback to the use of the regression technique (14). The correlation between age and weight and between age and body size in women made it impossible to draw conclusions directly from regressions on age as to how much of the dependence was on age if influences of weight and body surface area were eliminated. This could also influence on the comparison between the sexes as no similar relationship between age and body size was found in men. Analyses of the partial correlation coefficients were made for the more important variables to explore the effects of age independent of body size on these variables in women and of weight or body surface area independent of age. Such analysis of heart rate with age, weight and body surface area added no significant partial correlation coefficient. The systolic pressure and age independent of weight had no significant partial correlation coefficient (0.426) being at variance with the results reported by Truedsson (51). The coefficient with age independent of body surface area, however, remained significant (0.467, $P < 0.05$). Diastolic pressure and age independent of weight and body surface area had no significant correlations and the correlations between diastolic pressure and weight and body surface area independent of age were insignificant (0.355 and 0.315 respectively). This result of weight correlation when influence of age was eliminated was also at variance with the report of Truedsson (51). The significant correlation between mean pressure and age disappeared after adjustment for influence of weight and body surface area. The coefficients were 0.391 and 0.423 respectively after adjustment. No additional correlation between mean pressure and adjusted weight and body surface area was obtained.

Much of the influence of age on pressures in women was thus due to increase of weight and body surface area with increasing age.

Stroke volume, arteriovenous oxygen difference and pulmonary ventilation had no significant partial correlations with age, weight and body surface area in women.

Cardiac output had no significant partial correlation with age adjusted for weight or body surface area influence. The partial correlation coefficients between cardiac output and weight and body surface area adjusted for age influence were 0.467 ($P < 0.05$) and 0.514 ($P < 0.05$) respectively. The

partial correlation coefficients of systemic vascular resistance and age adjusted for weight and body surface area influence were 0 475 ($P < 0.05$) and 0 504 ($P<0.05$) respectively, but the correlations with weight and body surface area adjusted for age influence were insignificant

The correlations of left ventricular work with weight and body surface area remained after age adjustments The coefficients were 0 500 ($P < 0.05$) and 0 473 ($P < 0.05$) respectively after adjustment

The correlation coefficient between oxygen consumption and body surface area remained significant after age adjustment (0 528, $P<0.05$) but the correlation coefficient with weight was insignificant after age adjustment (0 441)

The nonrandom sampling hitherto employed in all hemodynamic studies where similar variables have been studied is a drawback when statistical inferences are to be made In addition the subjects were chosen according to the values which were to be analysed They had to be free from circulatory diseases Upper limits for the systolic and diastolic arterial pressures were also set

These obstacles were probably small The tests of normality showed no significant skewness or kurtosis of the distributions of systolic, mean or diastolic pressures in either sex Only for pulse pressure in men was a deviation found The only significant deviation from normality of skewness and kurtosis obtained among the variables calculated from mean arterial pressure was that of left ventricular work in men

Thus the material seemed suitable to study the effect of age, sex and body size on hemodynamic and respiratory parameters of adult normal subjects It was, however, a cross sectional study and gave no information on how ageing and change of body size in each subject affects the circulation and respiration as a longitudinal study could have given

With regard to the circulation and respiration, rest has been thought to be a less well defined functional state than exercise, since emotions are thought to be more disturbing at rest (53)

To evaluate the 'degree of rest the oxygen consumption was recalculated to basal metabolic rate (8) The mean values and standard deviations in men were + 8 7 and 11 0 % ($P <0.001$) and in women + 6 7 and 9 6 % ($P <0.01$) respectively These small but significant elevations indicated little disturbance of the resting state from the complicated procedure

The lack of dependence on age or body size of adult heart rate was in agreement with other studies (48) and this was also true for the lack of sex difference (51) Higher resting heart rate in women compared to men in adolescens has however, been reported (32) and also changing heart rate with age in women (13)

Only the women had an age dependence of arterial pressures The adjustments for the small sex difference of mean age did not change the sex relations of the systolic and mean arterial pressures The dependence on weight and body surface area in women of the diastolic pressure was mainly due to the

increasing body size with ageing according to the partial correlation analysis Age however had no significant influence in simple regression on diastolic pressure The sex differences of diastolic pressure after adjustments for weight and body surface area were thus hard to evaluate The sex difference of pulse pressure which was even more pronounced after adjustment for body size might have physiological reasons There may be other relations in men and women between stroke volume, its rate of ejection and the arterial system in qualitative (elasticity?) and quantitative respects These results are at variance with those obtained in a population study from India of adult men and women who had no correlation between weight and age and who had significant positiv correlation between systolic as well as diastolic pressure on one side and age and weight on the other side (13)

More important were the results of the flow dependent variables which were not so much influenced by the selection of the subjects The smaller cardiac output of women was associated with differences in body surface area This was in agreement with many other studies (for references see Wade and Bishop (53)) However, the dependence on body surface area was only significant in men and in the men, height was at least as good in predicting the cardiac output Adjustment using height also eliminated the sex difference of cardiac output When age was used for adjustment together with body surface area however the sex difference remained The problem of sex difference cannot be considered settled

The stroke volume had no significant dependence on height in men but on weight and body surface area Only adjustment using body surface area eliminated the sex difference

An explanation for the lack of dependence on body size of cardiac output and stroke volume in women might be found in a higher variability of the vascularization of the body in women More variation in amount of fat and muscle tissue which have a low blood flow during rest might be the reason No special measurements of the fat or muscle factors have, however been made However age influenced on this relationship as seen from the significant partial correlation between cardiac out put and body size independent of age

The relationship between body size and cardiac output was not as clear in the present study as in the study of Jegier *et al* (30) Their higher correlation might have been due to a higher span of body size Children from 3 weeks of age to adults of 52 years of age were included The correlation obtained by Smulyan *et al* (45) are more in agreement with the present material However they had a higher dependence on weight than on height for cardiac output Calculations using body surface area for indexing cardiac output seemed justified in men in the present study but not in women This also applied to stroke volume

To make an index with height was impossible due to the significant deviations from origin of the regression line

No single dependence on age was evident in this comparatively small sample in contrast to the results reported by Smulyan *et al* (45) and Brandfonbrener *et al* (9)

Oxygen consumption had also been considered to be well correlated to the cardiac output at rest (19) The regression in the present material of cardiac output (y) on oxygen consumption (x) in man was

$y - 1\ 87 + 0\ 0173\ x$ (t (b/s) $= 2\ 49$ $P < 0\ 025$ $r = 0\ 469$ $s_{yx} - 1\ 10$)

and in women

$y - 1\ 96 - 0\ 0169\ x$ (t (b/) $= 2\ 07$ $P < 0\ 10$ $r = 0\ 438$ $s\ _x = 0\ 80$) The last dependence was however only indicative

The calculated work of the left ventricle per minute and stroke had regressions in men on weight and body surface area In women the left ventricular work also had significant similar regressions Adjustment according to sex differences of the independent variables also eliminated the sex differences

The dependences of systemic vascular resistance and conductance on age and body size have been rarely explored The present sex difference of resistance could be explained by differences in height but not in body surface area The difference of conductance was eliminated only when height adjustment was made

Of the respiratory variables the oxygen consumption was higher in men also when the values were adjusted according to the dependences on weight and body surface area A smaller proportion in men of less oxygen consuming tissues during rest e g fat and muscle might be the reason In women the oxygen consumption was dependent on each of height and body surface area but also on weight which however was eliminated after partial correlation adjustment for age When these regressions were used for adjustments the sex difference remained The dependences on body surface area and weight in women were thus not identical with those in men

The higher variance of pulmonary ventilation in men remained also after adjustment for the only dependence on height found in men Similarly with the different variances of oxygen consumption which remained after adjustment for weight and body surface area

According to the present data indexing using body surface area seemed justifiable for oxygen consumption but not for pulmonary ventilation In men indexing using weight was inadmissible as the regression line did not pass through the intersection of the axis In women a better prediction was derived by using height instead of body surface area possibly due to the hypothetical influence of more variable distribution of tissues with low oxygen consumption

The usual sex difference in hematocrit was evident in this series However no regression on body size or age was found

The efficiency of the regression equations in predicting the dependent variable from the independent anthropometric variables was not great judged from the correlation coefficients Another expression for this is the decrease of the nonregression standard deviation

to the residual standard deviation of the regression equation The quotient between these gives the percentage increase in precision which ranged from 108 for predicting systemic vascular conductance in men from body surface area to 137 for predicting the left ventricular stroke work in men from weight

Summary

The approach using a linear regression technique to study the influence of age and body size on some circulatory and respiratory measurements in men and women during rest in recumbency has given significant regressions of most measurements on body size in men but only of a few on body size in women This was thought to be due to a more variable vascularization and metabolism in the body of women

Using the regressions for sex comparisons, differences of mean values for brachial arterial pressures, cardiac output stroke volume systemic vascular conductance and oxygen consumption were found

Age in women had influence on the arterial pressure and in partial correlation on systemic vascular resistance after adjustment for weight and body surface area Age in men had an indicative influence on cardiac output when age was combined with body surface area Certain influences on age combined with body surface area were also evident for systemic vascular resistance in men and for systemic vascular conductance in men and women

II Studies at rest in the sitting position

by G Schroder R Malmcrona &
R Sannerstedt

Most studies at rest have been performed with the subjects recumbent for references see Wade & Bishop (53) standing (23 41 52 55) or tilted to various degrees (33 52) There are also a few reports (4 20 31 35 55) on the hemodynamics in the sitting position

This paper presents the results of 59 men and 28 women investigated in the sitting position Multiple regression analysis of each sex was made to evaluate the influence of age and anthropometric measurements on the functions studied Sex comparisons were made before and after adjustments to the regressions found to determine if differences in age or body size were the reason for the sex differences obtained

Subjects

The criteria for accepting subjects into the study were the same as reported previously (part I)

There were 59 men between the ages of 17 and 59 and 28 women between the ages of 18 and 56 Some statistics on age and anthropometric data are given in table I and fig 1

The majority of the subjects 43 men och 21 women were paid volunteers from various occupations and social classes Sixteen men and seven women

Table I *A and anthropometric data in 59 men and 28 women studied at rest sitting in a la Mean values ($\bar{x}$) and standard deviation (s_x)*

	MEN		WOMEN	
	$\bar{x}$	s_x	$\bar{x}$	s_x
Age (years)	33 6	11 3	36 3	11 8
Height (m)	1 788	0 059	1 653	0 053
Weight (kg)	75 8	12 7	64 6	8 4
Body surface area (m²)	1 935	0 159	1 708	0 124

) Tables with age and anthropometric data and individual results on each subject can be obtained from the authors upon request

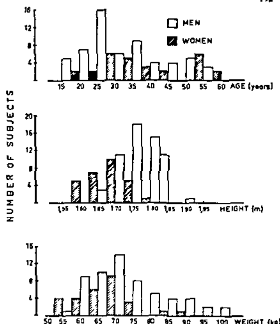


Fig 1 Histogram for distribution of age height and weight

were patients considered to have a normal circulatory system Ten of the men were in the late convalescence from peptic ulcer disease and five women were candidates for mammaplasty The methods of selection have been described (part I)

Thirty-six men and 19 women were classified as manual workers while 23 men and nine women had mainly sedentary work Nine men and one women were students and there were 16 housewives Regular physical training was reported by 17 men and four women

Performance

The experimental procedure followed the protocol described previously (part I)

The brachial artery and cubital vein were catheterized The subject rested for about half an hour sitting comfortably in an armchair after which the recordings were started The back of the chair was inclined 60 degrees from the horizontal plane The recordings included heart rate (ECG) intraarterial pressures cardiac output (dye dilution), pulmonary ventilation and oxygen consumption Zero level for pressure recor-

dings was placed horizontal to the third intercostal space Ventilatory values were from expired air Measurements at rest in the recumbent position preceded these determinations in 44 subjects (part I) The methods for obtaining the variables and the statistical methods for processing them are given in part I

Results

The mean values, standard deviations and P-values of difference according to t test are given in table II Linear regression equations on age and anthropometric measurement are given in table III and adjusted values to the mean values of anthropometric data and age of the other sex are given in table IV together with sex comparisons

Heart rate (HR)

The heart rate was not significantly different in men and women No regressions on age or body size were obtained in either sex No significant deviation from normal distribution of skewness (g_1) or kurtosis (g_2) was evident in either sex The variances were not different

Table II *Mean values ($\bar{x}$) standard deviations (s_x) and P values of differences between men and women of hemodynamic and respiratory variables in the sitting position For abbreviations see text*

		MEN		WOMEN		Sex differences of	
		$\bar{x}$	s_x	$\bar{x}$	s_x	Mean values P	Variances P
HR	beats/min	67 0	9 3	69 3	11 4	–	–
$P_{S_{BA}}$	mm Hg	124 5	10 7	124 8	10 4	–	–
$P_{D_{BA}}$	mm Hg	73 5	6 8	71 1	7 1	–	–
$\bar{P}_{BA}$	mm Hg	91 8	8 1	91 3	8 4	–	–
$P_{S_{BA}}-P_{D_{BA}}$	mm Hg	51 0	7 3	53 7	7 3	–	–
Q	l/min	6 23	1 07	5 28	0 82	<0 001	–
SV	ml	93 9	15 4	77 2	12 0	<0 001	–
LVW	kpm/min	7 79	1 60	6 54	1 08	<0 001	<0 05
LVSW	pm	117 1	21 9	96 0	17 8	<0 001	–
SVR	mm Hg/l/min	15 10	2 66	17 68	3 13	<0 001	–
SVC	ml/min/mm Hg	68 3	12 0	58 4	11 2	<0 001	–
VO_2 (STPD)	ml/min	278 3	32 4	225 5	27 6	<0 001	–
V_E (BTPS)	l/min	8 57	1 85	7 27	1 71	<0 001	–
R		0 805	0 066	0 810	0 083	–	–
$(a-\bar{v})O_2$		45 5	6 8	43 3	7 0	(<0 20)	–
Hct		42 0	2 94	37 3	3 65	<0 001	<0 05

Table III *Linear regressions of hemodynamic and respiratory variables (y) on the independent variables (x): age (years), height (m), weight (kg) and body surface area (m²) in 59 men (m) and 28 women (f) during rest sitting in a chair. When fewer are included due to computer program errors the numbers are in brackets. s_{yx} residual standard deviation; b regression coefficient; b/s_b t value for regression coefficient; r correlation coefficient*

Eq nr	y		x	Regression equation	s_{yx}	b/s_b	P	r
1	P_{SBA}	f	Age	$y = 108.3+0.453\,x$	9.12	3.05	<0.01	0.513
2	$\bar{P}_{BA}$	f	Age	$y = 81.2+0.279\,x$	7.85	2.18	<0.05	0.393
3	$\bar{P}_{BA}$	m	Age	$y = 84.9+0.203\,x$	7.87	2.22	<0.05	0.287
4	$P_{SBA} \cdot P_{DBA}$	f	Age	$y = 41.8+0.328\,x$	6.25	3.22	<0.005	0.533
5	Q	m	Age	$y = 7.21-0.0290\,x$	1.02	2.46	<0.02	0.308
6	Q	m	Height	$y = -4.93+6.244\,x$	1.01	2.76	<0.01	0.345
7	Q	m	BSA	$y = 2.41+1.972\,x$	1.03	2.31	<0.025	0.294
8	Q (57)	m	Age x_1	$y = 2.60-0.0287\,x_1$	0.98	2.50	<0.02	
			BSA x_2	$+2.38\,x_2$		2.57	<0.02	0.435
9	Q	m	Age x_1	$y = -2.50-0.231\,x_1$	0.98	2.03	<0.05	
			Height x_2	$+5.30\,x_2$		2.38	<0.025	0.470
10	SV	m	Age	$y = 106.8-0.385\,x$	14.9	2.22	<0.05	0.282
11	SV	m	Height	$y = -77.7+96.0\,x$	14.4	2.97	<0.005	0.366
12	SV	m	Weight	$y = 53.6+0.531\,x$	14.0	3.69	<0.001	0.439
13	SV	m	BSA	$y = 3.5+46.7\,x$	13.7	4.13	<0.001	0.480
14	SV	m	Age x_1	$y = 66.4-0.398\,x_1$	13.4	x_1 2.56	<0.02	
			Weight x_2	$+0.539\,x_2$		x_2 3.92	<0.001	0.576
15	SV (57)	m	Age x_1	$y = 4.7-0.376\,x_1$	13.2	2.44	<0.02	
			BSA x_2	$+57.8\,x_2$		4.23	<0.001	0.551
16	LVSW	m	Weight	$y = 62.0+0.726\,x$	20.1	3.54	<0.001	0.421
17	LVSW	m	BSA	$y = -5.7+57.5\,x$	20.1	3.43	<0.005	0.415
18	SVR	m	Age	$y = 11.50+0.107\,x$	2.39	3.86	<0.001	0.456
19	SVR	m	Height	$y = 52.88-21.13\,x$	2.37	4.00	<0.001	−0.467
20	SVR	m	BSA	$y = 25.99-5.625\,x$	2.52	2.68	<0.01	0.335
21	SVR	m	Age x_1	$y = 43.54+0.0876\,x_1$	2.18	3.38	<0.005	
			Height x_2	$17.56\,x_2$		3.52	<0.001	0.593
22	SVR (57)	m	Age x_1	$y = 24.86+0.106\,x_1$	2.20	4.12	<0.001	
			BSA x_2	$-6.92\,x_2$		3.34	<0.005	0.583
23	SVR	f	Age	$y = 13.08+0.127\,x$	2.80	2.78	<0.01	0.4[illegible]
24	SVC	m	Age	$y = 83.89-0.465\,x$	10.9	3.67	<0.001	0.422
25	SVC	m	Height	$y = -98.1+93.02\,x$	10.7	3.87	<0.001	0.456
26	SVC	m	BSA	$y = 22.4+23.71\,x$	11.5	2.49	<0.02	0.314
27	SVC	m	Age x_1	$y = 57.7-0.379\,x_1$	9.98	3.18	<0.005	
			Height x_2	$+77.58\,x_2$		3.40	<0.005	0.553

Eq nr	y		x	Regression equation	s_{yx}	b/s_b	P	r
28	SVC (57)	m	Age x_1	$y = 27.3 - 0\ 460\ x_1$	10 1	3 90	<0 001	
			BSA x_2	$+29.29\ x_2$		3 08	<0 005	0 558
29	SVC	f	Age	$y = 72\ 0 - 0.374\ x$	10 4	2 20	<0 05	−0.396
30	VO	m	Height	$y = -77\ 5 + 199\ 0\ x$	30 4	2 93	<0 005	0.361
31	VO_2	m	Weight	$y = 174\ 6 + 1.37\ x$	27 5	4 84	<0 001	0 537
32	VO_2	m	BSA	$y = 61.3 + 112\ 1\ x$	27 3	4 96	<0 001	0 548
33	VO	f	Height	$y = -206.3 - 261\ 2\ x$	24 4	2 94	<0 01	0 499
34	VO_2	f	Weight	$y = 119\ 5 + 1\ 640\ x$	24 4	2 94	<0 01	0 499
35	VO	f	BSA	$y = 18\ 2 + 121\ 4\ x$	23 6	3.32	<0 005	0 546
36	V_E	m	Height	$y = -12.74 - 11\ 92\ x$	1 73	3 07	<0 005	0.378
37	V_E	m	Weight	$y = 4\ 55 + 0\ 053\ x$	1 74	2 96	<0 005	0 365
38	V_E	m	BSA	$y = -0\ 45 + 4\ 66\ x$	1 72	3 28	<0 005	0.398
39	$(a-\bar{v})O_2$	f	Age	$y = 31\ 7 - 0\ 321\ x$	5 96	3.30	<0 005	0 543
40	$(a-\bar{v})O$	f	Weight	$y = 16\ 63 - 0\ 413\ x$	6 16	2 93	<0 01	0 498
41	$(a-\bar{v})O$	f	BSA	$y = 0\ 883 - 24\ 85\ x$	6 37	2 52	<0 02	0 443
42	Hct	f	Age	$y = 33\ 11 + 0\ 115\ x$	3 44	2 06	<0 05	0 374
43	Hct	f	Weight	$y = 26\ 22 + 0\ 171\ x$	3 40	2 21	<0 05	0.395

Brachial arterial pressures Brachial arterial systolic ($P_{S_{BA}}$) diastolic ($P_{D_{BA}}$) mean ($\bar{P}_{BA}$) and pulse pressure ($P_{S_{BA}} - P_{D_{BA}}$)

No significant differences in brachial arterial pressures were found between men and women

In men a regression of the brachial arterial mean pressure on age was found (3) In women the brachial arterial mean pressure also had a regression on age (2) The women also had regressions of the systolic pressure (1) and pulse pressure (4) on age Age-adjustment did not reveal any sex difference The regression equations of the dependence of the mean arterial pressure on age in both sexes were identical according to identity test No significant deviations of g1 and g2 from normality were evident in either sex for any pressure The variances of the pressures were not different in men and women

Flow dependent variables Cardiac output (Q), stroke volume (SV), left ventricular work (LVW) and stroke work (LVSW), systemic vascular resistance (SVR) and conductance (SVC)

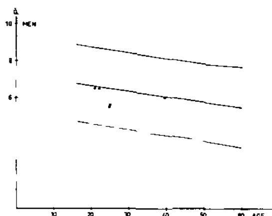
In men, the mean values for the cardiac output, stroke volume, left ventricular work and stroke work, and the systemic vascular conductance were higher than in women The systemic vascular resistance was lower

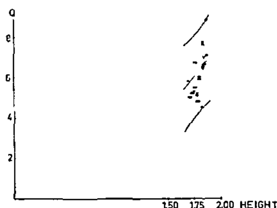
) Equation nr table III and IV

Table IV *Adjusted mean values ($\bar{Y}/x$) and residual standard deviations (s_{yx}) for the mean values of age and body size of the other sex. Comparisons were made with the mean values of the other sex. If regressions were found for that sex, the one with the lowest residual standard deviation was used for testing. For further abbreviation see the text*

Eq nr	Variable	Sex	Adjustment for	Adjusted values $\bar{Y}/x$	Adjusted values s_{yx}	Sex differences of mean values P
1	$P_{S_{BA}}$	f	Age	123 6	9 3	—
2	$\bar{P}_{BA}$	f	Age	90 5	8 0	—
3	I_{BA}	m	Age	92 4	7 9	—
4	$P_{S_{BA}}-P_{D_{BA}}$	f	Age	52 8	6 4	
5	Q	m	Age	6 15	1 03	<0 001
6	Q	m	Height	5 39	1 06	—
7	Q	m	BSA	5 78	1 06	<0 05
8	Q	m	Age BSA	5 63	1 01	—
9	Q	m	Age Height	5 45	1 04	—
10	SV	m	Age	92 9	15 0	<0 001
11	SV	m	Height	80 9	15 2	—
12	SV	m	Weight	88 0	14 2	<0 001
13	SV	m	BSA	83.3	14 1	(<0 10)
14	SV	m	Age Weight	86 8	13 6	<0 005
15	SV	m	Age BSA	81 3	13 6	—
16	LVSW	m	Weight	109 0	20 4	<0 01
17	LVSW	m	BSA	104 0	20 6	(<0 10)
18	SVR	m	Age	15 41	2 41	<0 001
19	SVR	m	Height	17 89	2 49	—
20	SVR	m	BSA	16 38	2 59	<0 05
21	SVR	m	Age Height	17 67	2.30	—
22	SVR	m	Age BSA	16 88	2.26	
23	SVR	f	Age	17 34	2 85	<0 001
24	SVC	m	Age	67 0	11 0	<0 001
25	SVC	m	Height	56 0	11 2	—
26	SVC	m	BSA	62 9	11 8	—
27	SVC	m	Age Height	57 0	10 5	
28	SVC	m	Age BSA	60 7	10 4	—
29	SVC	f	Age	59 4	10 6	<0 001
30	VO	m	Height	251 4	32 0	<0 001
31	VO	m	Weight	270 0	27 9	<0 001
32	VO	m	BSA	252 9	28 0	<0 001
33	VO	f	Height	260 8	27 6	<0 01
34	VO	f	Weight	243 9	25 6	<0 001

Eq nr	Variable	Sex	Adjustment for	Adjusted values $\hat{Y}/x$	Adjusted values s_{YX}	Sex differences of mean values P
35	$\dot{V}_O$	f	BSA	253.1	25.4	< 0.001
36	$\dot{V}_E$	m	Height	6.96	1.82	—
37	$\dot{V}_E$	m	Weight	7.98	1.77	(< 0.10)
38	$\dot{V}_E$	m	BSA	7.51	1.76	—
39	$(a-\bar{v})_O$	f	Age	42.4	6.1	< 0.05
40	$(a-\bar{v})_O$	f	Weight	47.9	6.5	—
41	$(a-\bar{v})_O$	f	BSA	48.9	6.9	< 0.05
42	Hct	f	Age	36.97	3.50	< 0.001
43	Hct	f	Weight	39.19	3.57	< 0.001





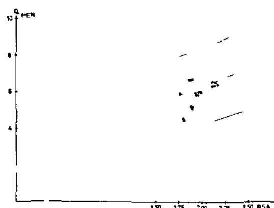


Fig. 2–4. Regression lines and 95 % confidence limits for the dependence of cardiac output (l/min) Q on age (yrs) 2) height (m) 3) and body surface area (m²) BSA 4) in men

Cardiac output of men had regressions on each of age (5) (fig 2) height (6) (fig 3) and body surface area (7˘) (fig 4) The adjusted values remained higher than the mean value for women except for the regression on height, where no difference remained after adjustment Cardiac output of men also had trivariate regressions on age and body surface area (8˘) and on age and height (9) The adjusted values were not significantly different from the mean value for women

The women had no regressions of significance of cardiac output on age or anthropometric data

The stroke volume of men had regressions on age (10˘) (fig 5) height (11) (fig 6), weight (12˘) (fig 7) and body surface area (13*) (fig 8) The adjusted values for age and weight were significantly higher than the mean value of women The difference of the adjusted value for body surface area was only indicative and no difference remained when adjustment was made for height difference The stroke volume of men had trivariate regressions on age and body surface area (15*) and on age and weight (14) and the adjusted value for age and weight remained higher but

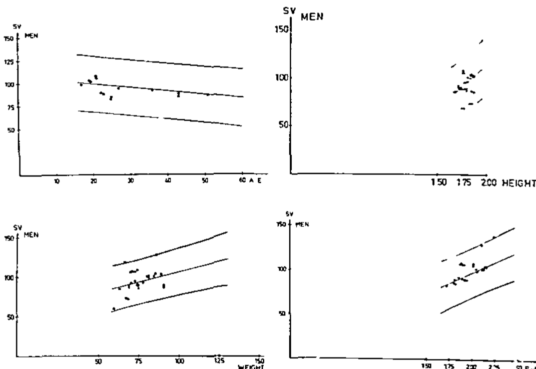


Fig 5 6 7 8 Regression lines and 95 % confidence limits for the dependence of the stroke volume (ml) SV on age (yrs) 5) height (m) 6) weight (kg) 7) and body surface area (m) 8) in men

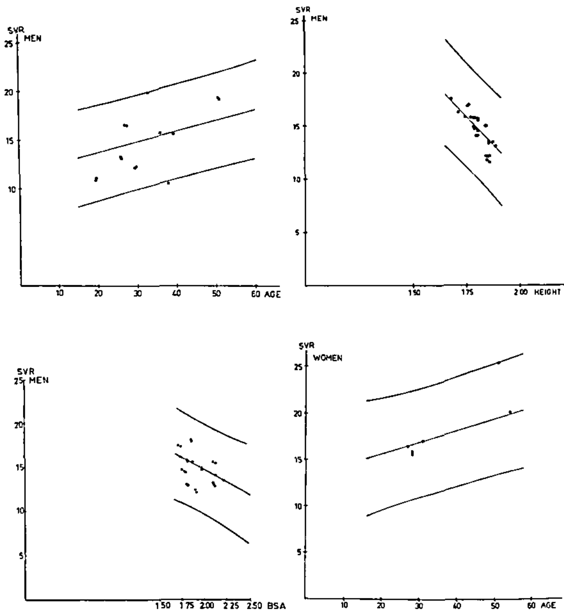


Fig 9 10 11 12 Regression lines and 95 % confidence limits for dependence of the systemic vascular resistance (mm Hg/l min) SVR on age (yrs) 9) height (m) 10) and body surface area (m) BSA 11) in men and on age 12) in women

the sex difference disappeared when adjustment was made for age and body surface area

The women had no regressions for stroke volume on age or anthropometric data

The left ventricular work values had no regressions in either sex The left ventricular stroke work of men had regressions on weight (16) and body surface area (17) The weight adjusted value was significantly higher than the mean value of women but the adjusted value for body surface area only had an indicative difference

The systemic vascular resistance of men had regressions on age (18*) (fig 9), height (19) (fig 10) and body surface area (20) (fig 11) as well as trivariate regressions on age and height (21*) and on age and body surface area (22) The sex difference remained for the age adjusted value and for the body surface area adjusted value The adjusted values for height for age and height and for age and body surface area were not significantly different from the mean value for women

The systemic vascular resistance of women had only a regression on age (23*) (fig 12), the adjusted value of which was higher than the mean value of men Identity test of these regressions in the two sexes thus rejected this hypothesis The regression coefficients, however, were not significantly different

The vascular conductance had regressions on age and on the same anthropometric data as the systemic vascular resistance (25 , 26*, 27*, 28 29*)

The age adjusted value for men remained higher but the adjusted values for height, for body surface area and for age and height, and for age and body surface area were not different from the mean conductance for women The age adjusted value for women was lower than the mean value of men Identity test of dependences on age of the regressions in men and women thus rejected this hypothesis The regression coefficients, however, were not significantly different

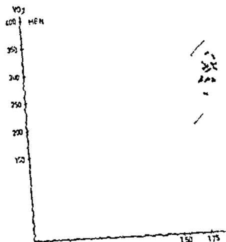
In men no significant deviations of g_1 and g_2 from normality were found for the flow dependent variables In women, the same was true for stroke volume and systemic vascular resistance, left ventricular work and stroke work The cardiac output had significant skewness and kurtosis ($g_1 = 3\ 35$ $P < 0\ 005$ $g_2 = 4\ 57$ $P < 0\ 001$) as had systemic vascular conductance ($g_1 = 2\ 70$, $P < 0\ 02$ and $g_2 = 2\ 87$, $P < 0\ 01$) Left ventricular work was the only flow dependent variable which had different variance between the sexes It was higher in men

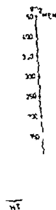
Respiratory variables Oxygen consumption ($\dot{V}o_2$), pulmonary ventilation ($\dot{V}_E$), ventilatory exchange ratio (R) and arteriovenous oxygen difference ($(a-\bar{v})o_2$)

The mean values of oxygen consumption and pulmonary ventilation were higher in men than in women No differences of the ventilatory exchange ratio and the arteriovenous oxygen difference were found

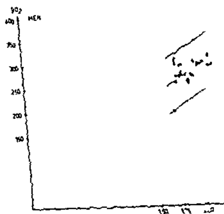
Regressions on each of height (30*) (fig 13) weight (31) (fig 14) and body surface area (32*) (fig 15) were found in men for the oxygen consumption The adjusted values were higher than the mean value for the women

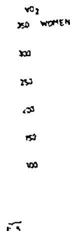
The women also had regressions on each of height (33) (fig 16) weight



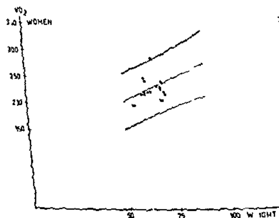












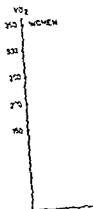


Fig 13 14 15 16 17 18 Regression lines with 95 % confidence limits for the ... oxygen consumption (ml min) VO₂ on height (m) 13) weight (kg) 14) and bod... (m²) BSA 15) in men and on height 16) weight 17) and body surface area 18) in wo...

10 1
resi
in n

x di
ment
area

(34) (fig 17) and body surface area (35') (fig 18) The adjusted values were lower than the mean value for men The coefficients of regressions were not significantly different

The pulmonary ventilation of men had regressions on each of height (36), weight (37) and body surface area (38) The adjusted values were not significantly different from the mean value for women The women had no significant regression of pulmonary ventilation The ventilatory exchange ratio had no regression in either sex

The arteriovenous oxygen difference in women had regressions on each of age (39), weight (40) and body surface area (41) The adjusted values of age and body surface area were significantly different from the mean value for men being lower and higher, respectively The weight adjustment revealed no difference The men had no regression for the arteriovenous oxygen difference

Oxygen consumption had no deviations of g_1 and g_2 from normality in either sex Pulmonary ventilation, however, had significant skewness and kurtosis in both sexes (men $g_1 = 1\ 43$, $P < 0\ 001$, $g_2 = 3\ 35$ $P < 0\ 001$ and women $g_1 = 2\ 43$, $P < 0\ 001$, $g_2 = 5\ 53$, $P < 0\ 001$) Arteriovenous oxygen difference had no significant skewness or kurtosis in men but a skewness in women ($g_1 = 0\ 99$, $P < 0\ 05$)

The ventilatory exchange ratio had skewness in men ($g_1 = 1\ 29$, $P < 0\ 001$) and kurtosis in both sexes (men $g_2 = 2\ 57$, $P < 0\ 001$ and women $g = 2\ 05$, $P < 0\ 025$)

Variances were not different in men and women

Hematocrit (Hct)

The hematocrit value of women was lower than that of men No regressions on age or anthropometric data were found in men Women had regressions on each of age (42) and weight (43) These sex differences remained after adjustments

The variance of hematocrit was higher in women No deviation from normality of g_1 and g was found in either sex of the distribution of hematocrit

Discussion

The purpose of the study was to explore the effect of age and certain body dimensions on circulation and respiration of healthy individuals in the sitting position The age and body size influence on sex differences of the variables could thus also be analysed The effect of change from recumbency to sitting of the 44 subjects who were first studied in the lying position is analysed in another paper (part III)

The investigation was carried out on a selected material and the influence of this method on those inferences that can be drawn concerning the hypothetical population has been discussed (part I)

The distribution of age, weight, height and body surface area in women did not deviate from normality when skewness and kurtosis were considered (fig 1) No deviations from normality of age and height distribution in men were found but the distribution of weight and body surface area had skewness ($g_1 = 1\ 61$, $P < 0\ 001$ for weight and $g_1 = 0\ 80$, $P < 0\ 02$ for body surface area) The weight also had a kurtosis ($g_2 = 4\ 27$, $P < 0\ 001$) (fig 1) These deviations were mainly due to two overweight subjects (130 8 and 100 5 kg) Transformations, e g logarithm transformation to obtain more normal distributions were not tried The plots did not indicate that much gain could be achieved by transformations or by using quadratic or cubic expressions of the independent variables

The men had no regressions of height, weight or body surface area on age but women had a regression of weight (y) on age (x) ($y = 0\ 448\ x + 48\ 4$, $t\ (b/s_b) = 4\ 14$, $P < 0\ 001$, $s_{yx} = 6\ 66$ and $r = 0\ 629$) and another regression for body surface area (y) on age (x) ($y = 0\ 00599\ x + 1\ 4905$, $t\ (b/s_b) = 4\ 21$, $P < 0\ 001$, $s_{yx} = 0\ 1041$ and $r = 0\ 569$)

These regressions were similar to those of the subjects in the recumbent position (part I)

The correlations of age and weight and of age and body surface area in women made it uncertain to what extent the influences were of pure age, weight and body surface area when a significant regression was obtained with one of these independent variables The technique of partial correlation was used to obtain the adjusted correlation e g age and the variable studied after adjustment for the influence of weight or body surface area (10)

Analysis of heart rate and age adjusted for body surface area and weight disclosed no significant correlation Neither was any correlation between heart rate and adjusted body surface area or weight obtained The correlation coefficient between systolic pressure and age adjusted for body surface area was 0 501 ($P < 0\ 01$) and systolic pressure and age adjusted for weight 0 469 ($P < 0\ 05$) The systolic pressure had no significant correlations with weight and body surface area adjusted for age The correlation coefficients between mean pressure and age adjusted for weight and body surface area were not significant, 0 323 and 0 359 respectively

No significant partial correlation coefficients were obtained for cardiac output or stroke volume

The partial correlation coefficients between *systemic vascular resistance and* age adjusted for weight and body surface area were 0 449 ($P < 0\ 05$) and 0 506 ($P < 0\ 01$) respectively

The partial correlation coefficients between weight and body surface area adjusted for age and oxygen consumption also remained significant, 0 404 ($P < 0\ 05$) and 0 467 ($P < 0\ 05$) respectively

The pulmonary ventilation had no significant partial correlation coefficient and the arteriovenous oxygen difference had only one, age adjusted for body surface area, 0 395 ($P < 0\ 05$) Weight and body surface area adjusted for age and arteriovenous oxygen difference

had no significant partial correlation coefficient The same disappearances of the correlations were obtained for age and weight adjusted for each other and hematocrit

The partial correlation analysis thus reduced the number of significant correlations and added none

The influence of the deviations from normality in the men for weight and body surface area could possibly have caused some losses of correlations but such an influence is not great (14)

The degree of rest was hard to define properly The rather complicated procedure could have made the subjects anxious To get some information about this the oxygen consumption was recalculated to basal metabolic rate (8) The mean values were a little higher than the predicted values Mean value and standard deviation for men were +7 1 and 10 8 % ($P<0\ 001$) and for women +10 1 and 10 7 % ($P<0\ 001$), respectively These mean values were not different from the mean values in the recumbent position (part I) This small difference indicated that no great deviation from the basal conditions existed

The selection of the subjects according to level of arterial pressure caused no deviations from normality of skewness or kurtosis The regressions of systolic mean and pulse pressures on age for *women were also found in this curtailed* material In men only the mean pressure had this age dependence Dependences on body size measurements similar to those in the recumbent position were not obtained (part I) As expected arterial pressures had no sex differences

Concerning the dependences of the cardiac output in men, those on age combined with body surface area and age combined with height had the smallest residual standard deviations When single independent variables were considered, height was at least as good as body surface area in predicting the cardiac output These results were similar to the values obtained in the supine position from a large series including data from the literature reported by Smulyan *et al* (45) The weight dependence was, however not evident in the present study The method for cardiac index, using body surface area was admissible in the present series since the intersection with the y axis did not deviate significantly from the origin Height however, could not be used due to an intersection below the origin

The stroke volume had regressions on almost the same independent variables as the cardiac output for men and also one dependence on weight The lack of dependence of heart rate on age or body size measurements might have been the cause A lack of dependence of heart rate on age has been reported in adults (48) Disappearance of the sex difference of cardiac output and stroke volume after adjustment for a single independent variable was found only for height When age and height or age and body *surface area were combined as independent* variables the sex differences disappeared Thus there were no sex differences other than those which could be ascribed to differences in body size of men and women The lack of dependence on age or body size of cardiac

output and stroke volume in women could have been caused by a more variable body vascularization in women due to a more heterogeneous distribution of tissues, i e muscles and fat

The gains in precision from regressions, i e the decreases of standard deviation caused by the regressions, were slight On body surface area, the gains for cardiac output and stroke volume were 104 and 112 %, respectively as judged from the non regression standard deviation over the residual standard deviation

No dependence of cardiac output (y) on oxygen consumption (x) was obtained for women In men the regression equation was $y = 2\ 28 + 0\ 0142\ x$, $s_{yx} = 0\ 97$, $t\ (b/s_b) = 3\ 66$ $P < 0\ 001$ and $r = 0\ 432$

Sex differences for left ventricular stroke work were not completely eliminated after adjustment for differences in body size A higher proportion of less vascularized tissues in women might have been responsible for this difference and that for systemic vascular resistance When age was also adjusted for, however, the sex difference was eliminated

The gains in precision due to regressions of the flow dependent variables were not high The highest gains in men were for cardiac output, stroke volume, left ventricular stroke work, systemic vascular resistance and conductance 109, 115, 112, 122 and 120 %, respectively The corresponding figures for women of resistance and conductance were 112 and 108 % respectively

The slight skewness and kurtosis found in women for cardiac output and conductance probably had little influence on the regression analysis It was not thought to have caused loss of any correlations

The oxygen consumption difference in men and women was not eliminated when differences in body size were considered This also speaks in favour of a higher proportion of more oxygen consuming tissues in men at rest The sex difference of pulmonary ventilation, however, was eliminated when the differences in height and body surface area were taken into account

This might be explained by the higher variability of pulmonary ventilation as compared to oxygen consumption, as seen from the coefficients of variation In men these were 21 3 and 11 3 % respectively

The highest gains in precision of the regressions in men and women for oxygen consumption were 119 and 117 % respectively The corresponding figure for pulmonary ventilation in men was only 108 %

The higher arteriovenous oxygen difference in the sitting older men found by Granath *et al* (19) was not evident for men in the present study, but was for women Age adjustment revealed a higher arteriovenous oxygen difference in men This difference, however, was reversed after adjustment for body surface area difference The physiological importance of these relationships is questionable The only partial correlation coefficient of significance was between age and arteriovenous oxygen difference in women

Summary

The influence of age and body size on the circulation and respiration of normal men and women has been studied at rest in the sitting position. Despite the upper limit of arterial pressures selected in this study, regressions of pressures on age were obtained in both sexes. Body size had no influence on pressures. No sex differences of pressures were obtained.

The flow dependent variables, cardiac output, stroke volume as well as systemic vascular resistance and conductance in men were dependent on age as well as on body size and the lowest residual standard deviations were obtained when influences of age and body size were combined. In women resistance and conductance were the only flow dependent variables which had regressions. They were on age for both. The best regression equation eliminated the sex differences for the flow dependent variables.

For oxygen consumption, dependences were on body size variables in both sexes but none on age. The higher oxygen consumption in men remained when adjustments according to the regressions were made. The pulmonary ventilation in men but not in women had dependences on body size but not on age. The dependence could explain the sex difference.

Only in women, was any dependence for arteriovenous oxygen consumption found, which was on age as well as on body size. Before adjustments, no sex difference was obtained but after adjustments differences in both directions were found.

A higher proportion of less oxygen consuming and vascularized tissues in women was thought to contribute to the sex differences.

Age and weight as well as age and body surface area of women had significant correlations. Partial correlation analysis reduced the number of significant correlations between age, weight and body surface area and the hemodynamic and respiratory variables.

III Changing from recumbent to sitting position

Postural changes of hemodynamic measurements have been studied by many workers This has been reviewed by Wade & Bishop (53) and recent data have been reported by Tuckman & Shillingford (52), Ward *et al* (55) and Hanson *et al* (23) Sex differences and influence of age and body size on these responses are little studied

In this paper, an analysis of hemodynamic and respiratory responses of 24 men and 20 women in the seated and recumbent position will be given with sex comparisons Linear regression analysis of each sex is used to evaluate the influence of age and body size

Subjects

The method of accepting the subjects for the study and a closer presentation of the subjects have already been presented (part I) Statistics of age and anthropometric data together with an analysis of the distributions were given in part I

Performance and methods

Details of the procedure have been given (part I—II)

Catheters were placed in the brachial artery and in the right atrium or in a central vein After about half an hour s rest a set of recordings was made in the recumbent position The measurements included intraarterial pressures, pulse rate collection of expired air and during this collection, hematocrit and cardiac output The subject was seated in an armchair, with the back inclined at 60 degrees to the horizontal plane for another half an hour The measurements were repeated

The techniques for obtaining the variables have been described as well as the statistical and data processing methods (part I—II) Differences of coefficients of correlations have been tested according to the method using z-transformation (10)

*) Tables with the individual data are available from the author on request

Results

Mean values, standard deviations and probability of differences according to t test are given in table I. Linear regression equations with the value in the recumbent position as the independent variable and the corresponding value in the sitting position as the dependent variable are given in table II. The multiple regressions, after adding significant dependence on age and body size as independent variables, are given in table III.

Heart rate (HR)

No significant positional change of heart rate was obtained in either men or women nor was any change between men and women evident. No sex difference of the coefficients of correlation between the heart rates in the sitting and recumbent position was evident (1, 2)* (fig. 1 and 2).

Multiple regression analysis of the women disclosed dependences on age (33) and height (34) of the positional

Table I. *Mean values ($\bar{x}$), standard deviations (s_x) and probability values (P) of differences of hemodynamic and respiratory data at res[t] in the sitting position and the changes from the recumbent to the sitting position (positional change = sitting valu[e] — recumbent value) in 24 men and 20 women. For abbreviations see text*

	MEN					WOMEN						
	Sitting		Positional change			Sitting		Positional change			Sex differences of positional reaction (P)	
	$\bar{x}$	s_x	$\bar{x}$	s_x	P	$\bar{x}$	s_x	$\bar{x}$	s_x	P	Mean val.	Varian[ce]
HR beats/min	66.2	10.7	+ 1.9	6.3	–	68.2	9.8	–0.4	5.9	–	–	–
$P_{S_{BA}}$ mm Hg	121.8	12.7	– 2.0	6.4	–	126.2	10.8	–2.9	4.0	<0.005	–	<0.05
$P_{D_{BA}}$	72.4	6.7	– 0.3	4.8	–	71.7	7.4	–1.5	3.7	–	–	–
$\bar{P}_{BA}$	89.6	8.3	– 0.04	5.6	–	92.2	8.8	–1.9	3.2	<0.020	–	<0.01
$P_{S_{BA}}$ $P_{D_{BA}}$ mm Hg	49.4	8.4	– 1.8	4.1	<0.05	54.5	7.3	–1.5	3.6	–	–	–
Q l/min	6.071	0.958	– 0.754	0.732	<0.001	5.090	0.615	–0.595	0.697	<0.005	–	–
SV ml	93.9	19.5	–13.8	15.5	<0.001	75.4	10.1	–8.3	8.4	<0.001	–	<0.01
LVW kpm/min	7.40	1.42	– 0.88	1.05	<0.001	6.38	1.00	–0.88	0.90	<0.001	–	–
LVSW pm	114	26	–16	20	<0.001	95	18	–12	10	<0.001	–	<0.01
SVR mm Hg/l/min	15.09	2.72	+ 1.58	1.71	<0.001	18.37	2.94	+1.41	2.30	<0.020	–	–
SVC ml/min/mm Hg	68.2	11.4	– 8.3	8.6	<0.001	55.6	8.5	–5.3	7.9	<0.010	–	–
V_{O_2} ml (STPD)/min	291.6	36.0	+ 6.0	19.5	–	227.5	26.0	+6.6	18.1	–	–	–
V_E l (BTPS)/min	9.43	2.30	+ 0.16	2.51	–	7.11	1.52	+0.16	0.85	–	–	<0.01
R	0.818	0.092	– 0.027	0.089	–	0.796	0.078	–0.022	0.066	–	–	–
$(a-\bar{v})O_2$ ml/l	48.7	6.3	+ 5.9	5.5	<0.001	45.2	7.0	+5.8	6.4	<0.001	–	–
Hct	42.37	3.13	+ 0.67	0.86	<0.001	38.50	3.12	+0.10	0.64	–	<0.020	–

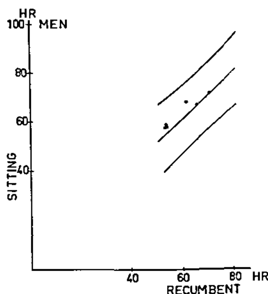
*) Eq. nr. from table II and III

Table II *Linear regressions of hemodynamic and respiratory variables in the sitting position (y) on the corresponding variables in the recumbent position (x) in 24 men (m) and 20 women (f)* s_{yx} *residual standard deviation* b *regression coefficient* s_b *the standard deviation of the regression coefficient* $\frac{b}{s_b}$ = *t value of regression coefficient* r *correlation coefficient For further abbreviations see table I*

Eq nr	y		Regression equation	s_{yx}	$\frac{b}{s_b}$	P	r
1	HR	m	y = 3 6 +0 973 x	6 5	6.38	<0 001	0 805
2	HR	f	y = 17 8 +0 735 x	5 2	7 14	<0 001	0 857
3	P_{SBA}	m	y = 10 0 +0 903 x	6 5	8 21	<0 001	0 868
4	P_{SBA}	f	y = 0 8 +0 972 x	4 1	10 81	<0 001	0 930
5	P_{DBA}	m	y = 14 8 +0 792 x	4 7	5 62	<0 001	0 730
6	P_{DBA}	f	y = −6 1+1 064 x	3 8	7 48	<0 001	0 867
7	$\bar{P}_{BA}$	m	y = 19 0 +0 788 x	5 4	5 89	<0 001	0 632
8	$\bar{P}_{BA}$	f	y = −5 5+1 039 x	3 3	10 72	<0 001	0 930
9	$P_{SBA}-P_{DBA}$	m	y = 3 1 +0 906 x	4 2	8 43	<0 001	0 874
10	$P_{SBA}-P_{DBA}$	f	y = 1 1 +0 954 x	3 7	7 51	<0 001	0 870
11	Q	m	y = 1 79+0 627 x	0 69	6 28	<0 001	0 801
12	Q	f	y = 2 66+0 428 x	0 50	3 21	<0 005	0 603
13	SV	m	y = 26 1 +0 629 x	13 2	5 32	<0 001	0 750
14	SV	f	y = 23 0 +0 626 x	7 5	4 04	<0 001	0 689
15	LVW	m	y = 2 10+0 640 x	0 85	6 47	<0 001	0 809
16	LVW	f	y = 2 32+0 559 x	0 73	4 27	<0 001	0 734
17	LVSW	m	y = 41 9 +0 545 x	20 2	3 92	<0 001	0 640
18	LVSW	f	y = 5 6 +0 834 x	9 6	7 12	<0 001	0 859
19	SVR	m	y = 3 81+0 835 x	1 69	6 14	<0 001	0 794
20	SVR	f	y = 7 42+0 646 x	2 03	4 68	<0 001	0 740
21	SVC	m	y = 20 4 +0 625 x	7 4	5 68	<0 001	0 770
22	SVC	f	y = 22 0 +0 552 x	6 7	3 61	<0 005	0 648
23	$\dot{V}O_2$	m	y = 29 4 +0 918 x	19 8	7.34	<0 001	0 844
24	$\dot{V}O_2$	f	y = 40 5 +0 847 x	18 3	4 53	<0 001	0 730
25	V_E	m	y = 5 24+0 452 x	1 80	3 93	<0 001	0 641
26	V_E	f	y = 1 30+0 836 x	0 84	6 71	<0 001	0 845
27	R	m	y = 0 398+0 495 x	0 075	3 60	<0 005	0 598
28	R	f	y = 0 327+0 576 x	0 048	5 48	<0 001	0 791
29	$(a-\bar{v})O_2$	m	y = 28 1 +0 490 x	4 8	3 27	<0 005	0 671
30	$(a-\bar{v})O_2$	f	y = 20 8 +0 620 x	6 2	2 48	<0 025	0 505
31	Hct	m	y = −1 2+1 045 x	0 9	16 4	<0 001	0 961
32	Hct	f	y = 0 5 +0 985 x	0 6	20 8	<0 001	0 978

Table III *Multiple regression analysis of hemodynamic and respiratory variables in the sitting position (y) on the corresponding variables in the recumbent position (x_1) and age or anthropometric data (x_2) (x_1 and x_2 — independent variables) For abbreviations see table I and II*

Eq nr	y		x_2	Regression equation	$s_{yx_1x_2}$	$\frac{b}{sb}$	P	r
33	HR	f	Age	$y = 27.4 + 0.727 x_1 - 0.231 x$	4.4	$x_1 = 8.30$ $x = 2.87$	<0.001 <0.020	0.907
34	HR	f	Height	$y = 82.2 + 0.705 x_1 - 38.0 x$	4.6	$x_1 = 7.60$ $x_2 = 2.41$	<0.001 <0.050	0.897
35	SVR	f	Age	$y = 6.03 + 0.550 x_1 + 0.0767 x_2$	1.85	$x_1 = 4.10$ $x_2 = 2.11$	<0.001 <0.050	0.801
36	V_E	m	Weight	$y = 1.33 + 0.397 x_1 + 0.0571 x_2$	1.59	$x_1 = 3.84$ $x_2 = 2.73$	<0.001 <0.020	0.751
37	V_E	m	BSA	$y = 3.25 + 0.377 x_1 + 4.68 x_2$	1.61	$x_1 = 3.53$ $x = 2.54$	<0.001 <0.020	0.740



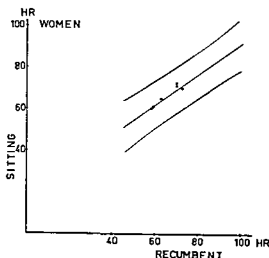


Fig 1 and 2 Regression lines and 95 % confidence limits of heart rate HR (beats/min) in sitting position (y) on the preceding determination in the recumbent position (x) of men (1) and women (2)

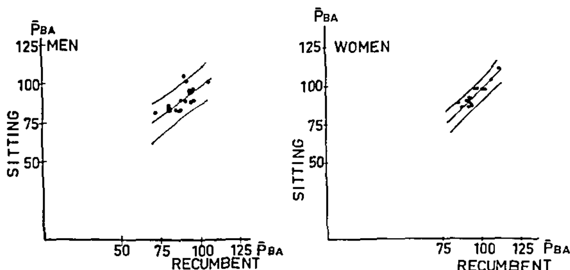


Fig 3 and 4 Regression lines and 95 % confidence limits of brachial arterial mean pressure $\bar{P}_{BA}$ (mm Hg) in the sitting position (y) on the preceding determination in the recumbent position (x) of men (3) and women (4)

response of heart rate The increases of precision due to these regressions on age and height judged from the quotient, standard deviation before regression over residual standard deviation after regression were 118 and 112 %, respectively The adjusted values to the mean age and mean weight of men were 69 5 and 63 7 beats per minute respectively in the sitting position When these values were used for comparisons no sex difference was evident The coefficients of skewness (g_1) and kurtosis (g_2) of heart rate in either sex or position did not deviate significantly from the normal distribution

Brachial arterial pressures Brachial arterial systolic ($P_{S_{BA}}$), diastolic ($P_{D_{BA}}$), mean ($\bar{P}_{BA}$) and pulse pressure ($P_{S_{BA}} - P_{D_{BA}}$)

In men a positional change affected only the pulse pressure which decreased in the sitting position In women, the systolic and mean pressure were lower in the sitting position However, no change of diastolic and pulse pressure in women was evident There was no difference of positional response of pressures between sexes

A high dependence of all brachial artery pressures in the sitting position on the corresponding measurements in the recumbent position was evident in both sexes (3—10)' (fig 3, 4) In women, the correlation coefficient (0 930) between the mean pressures in the two positions was higher than the corresponding coefficient (0 632) for men ($P<0\ 005$)

The positional responses of pressures had no dependence on age or body size. A skewnesss of the distribution for pulse pressure in the recumbent position of men ($g_1 = 2.61$, $P < 0.02$) was the only significant deviation from the normal distribution found for pressures.

The variances of positional change for systolic and mean pressure were higher in men than in women.

Flow dependent variables. Cardiac output (Q), stroke volume (SV), left ventricular work (LVW), left ventricular stroke work (LVSW), systemic vascular resistance (SVR) and systemic vascular conductance (SVC).

A decrease of cardiac output when seated was observed in men and women. The degree and variability of this response were not different in men and women.

The dependence of the seated cardiac output on the recording in recumbency had no sex difference (11, 12)* (fig. 5, 6).

The stroke volume was smaller in the sitting position and the decreases in men and women were not different. The variability of the response was, however, higher among the men. The variability of the stroke volume in the sitting position was also higher among the men.

The dependence of the sitting stroke volume on the corresponding recumbent value was not different between sexes (13, 14)* (fig. 7, 8).

The calculated values for left ventricular work and stroke work paralleled the changes of cardiac output and stroke volume (15—18)*.

The systemic vascular resistance increased to the same degree when seated in both sexes, nor was the variability of this response different. The predictability of the seated systemic vascular resistance from the corresponding value in the recumbent position was not different for the sexes (19, 20)* (fig. 9, 10). The only variable calculated from flow that had a dependence on age was systemic vascular resistance (35)*. None of the positional responses was dependent upon body size. The age dependence was evident only for women in whom the resistance increased when sitting up with 0.77 mm Hg/l/min for every additional decade of age. The increase of precision due to this age dependence was rather small, 11.0 %. The age adjusted resistance in the sitting position was 17.93 mm Hg/l/min which did not alter the differences discussed.

The systemic vascular conductance decreased to a similar extent in men and women upon sitting up. The predictability from the recumbent variables was also not different (21, 22)*. However, no dependence on age was found in either sex.

The only deviations from the normal distribution of the flow dependent variables were skewness ($g_1 = 2.24$, $P < 0.05$) and kurtosis ($g_2 = 2.26$, $P < 0.02$) of left ventricular work for men in the recumbent position.

Respiratory variables. Oxygen consumption (V_{O_2}), pulmonary ventilation (V_E), ventilatory exchange ratio (R) and arteriovenous oxygen difference ($(a-\bar{v})_{O_2}$).

No change in oxygen consumption

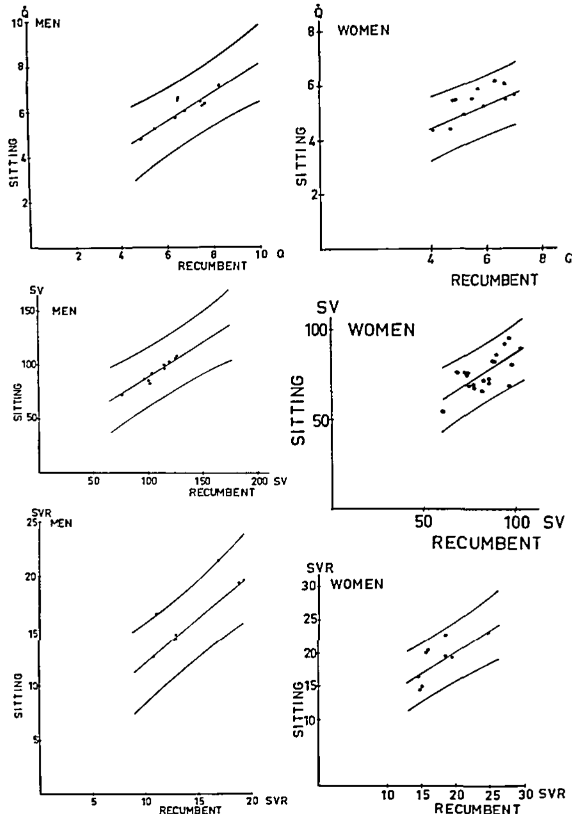


Fig 5 6 7 8 9 and 10 Regression lines and 95 % confidence limits of cardiac output Q (l/min) stroke volume SV (ml) and systemic vascular resistance SVR (mm Hg/l/min) in the sitting position (y) on the preceding determination in the recumbent position (x) of men (5 7 9) and women (6 8 10)

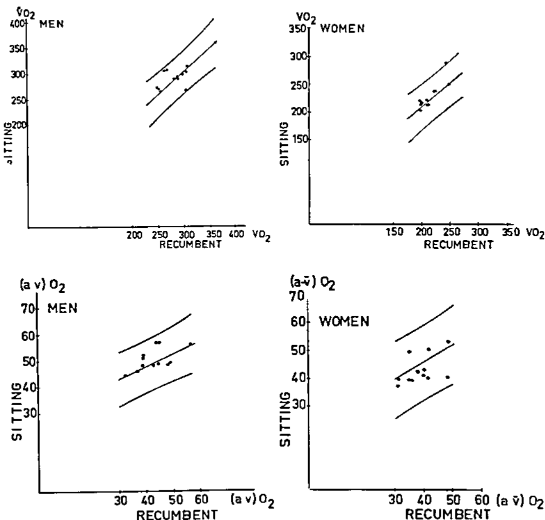


Fig 11 12 13 and 14 Regression lines and 95 % confidence limits of oxygen consumption $\dot{V}_{O_2}$ (ml/min) and arteriovenous oxygen difference $(a\ \bar{v})_{O_2}$ (ml/l) in the sitting position (y) on the preceding determination in the recumbent position (x) of men (11 13) and women (12 14)

was observed from the recumbent to seated position The predictability from the recumbent value was not different in men and women (23, 24) (fig 11, 12) No regressions for dependence on age or body size were found for the positional response The variability of the positional response had no sex difference

The pulmonary ventilation did not change in seated position and the predictabilities from the recumbent values

were not different in men and women (25, 26)*. The variance of the positional response was higher in men.

The pulmonary ventilation of men in the sitting position had dependences on weight (36)* and body surface area (37)* in addition to the dependence on the corresponding recumbent value. These dependences increased the precision to 113 and 112 % respectively. The adjusted seated pulmonary ventilations to the mean weight and body surface area of women were 8.84 and 8.13 l/min respectively.

Ventilatory exchange ratio did not change when seated in either sex. The variability of response as well as predictability from the recumbent value was not different between sexes (27, 28)*.

The arteriovenous oxygen difference when seated increased in both sexes (fig. 13, 14). This rise and its variability had no sex difference. The dependence on the corresponding recumbent variable was not different (29, 30)*.

Hematocrit (Hct)

The hematocrit in seated men increased but not in women. The predictability from the corresponding recumbent values was not different for the sexes (31, 32)*. No regression on age or body size was found for these changes. The variance of the positional response had no sex difference.

Deviation from the normal distribution of skewness and kurtosis for hematocrit was not found. Considering the respiratory variables, the pulmonary ventilation of men in the recumbent position had a skewness ($g_1 = 4.51$, $P < 0.001$) and kurtosis ($g_2 = 5.45$, $P < 0.001$). The skewness remained in the sitting position ($g_1 = 2.31$, $P < 0.005$). The seated values for women also had skewness ($g_1 = 5.52$, $P < 0.001$) and kurtosis ($g = 8.73$, $P < 0.001$). No other significant deviations of skewness or kurtosis were found.

Discussion

The dependences of weight and body surface area on age in women (part I) could have influence on the regressions for heart rate and systemic vascular resistance on age. Partial correlation analysis, however, has shown that the disturbance was not great (part I).

In men no such regressions of body size on age were found. They, however, had skewness and kurtosis of the weight distribution.

These circumstances as well as the non-random selection of the subjects are not thought to be a serious drawback to the present analyses.

The deviations from the normal distribution of skewness and kurtosis for the circulatory and respiratory variables were fewer in the sitting than in the recumbent position. They were observed in sitting only for pulmonary ventilation.

The unchanged heart rate upon sitting up from recumbent position was in agreement with the results obtained for

recumbent volunteers sitting up on a bicycle (18) Increased heart rate upon sitting on a bicycle (6) and upon sitting up in a chair (55) has been reported When the positional change was made by tilting the subjects 30—60 degrees on a table, the heart rate in a group of men and women also increased (52) The age dependence seen in women for the positional response can be compared with a similar response in men in which the heart rate increased when standing up This increase was lower at higher age (23, 48) In the present study, however, that response with increasing age of men could not be verified No age dependence of heart rate response of men tilted 45° head up was detected by Lee *et al* (34) The mechanism behind the dependence of the positional heart rate response on height in women is difficult to explain The opposite result an increase in heart rate in taller subjects, due to a greater redistribution of blood was in fact the expected finding

Concerning the brachial arterial pressures in men, only pulse pressure changed An unchanged mean arterial pressure of men and women seated in a chair from recumbent position has been reported by Ward *et al* (55) The decrease of diastolic pressure reported when men were seated on a bicycle (18) was not obtained in the present study Comparisons however are open to criticism as different reference levels for pressure were used The decrease of pulse pressure in men, was small as were the decreases of systolic and diastolic pressures in women when seated The relatively higher blood loss from the previous cardiac output determination in women was not thought to have influenced this response

The decrease of cardiac output in sitting position was in accordance with many studies in which the recumbent flow has been compared with the flow in up-right position (49 52 53, 55) The stroke volume change was responsible Concomitant decrease of the calculated left ventricular work and an increase of the calculated systemic vascular resistance was found since the arterial pressure was essentially unchanged

The multiple regression analysis showed a dependence for the systemic vascular resistance in women in the sitting position on age combined with the corresponding recumbent measurement Small changes in opposite directions of cardiac output and brachial arterial mean pressure must have been responsible for this In men no age dependence was found of systemic vascular resistance response The same result was obtained by Lee *et el* (34), when men were tilted 45° head up from the supine position

No increase of oxygen consumption when seated in a comfortable chair was observed It seemed to be more strenuous to sit on a bicycle waiting for an exercise test than in a chair as judged from the increase of oxygen consumption reported by Granath *et al* (18)

No pulmonary ventilation response resulted when seated as judged from the mean values This response however, depended on body dimensions being higher for heavier men and also for

larger men This might imply that pulmonary ventilation was more hampered in the recumbent position in those men compared with lighter men who probably had a smaller proportion of adipose tissue

The increase of arteriovenous oxygen difference when seated, was in accordance with similar studies (for references see Wade & Bishop (53))

The correlations between the recumbent and sitting recordings were high and only one sex difference of these correlations was found The mean pressures were better correlated in women than in men

Summary

The responses when changing from the recumbent to sitting position of heart rate, brachial arterial pressures, cardiac output oxygen consumption pulmonary ventilation and variables calculated from these measurements were analysed in 24 men and 20 women Influence of age, height weight and body surface area were studied by regressions analyses Sex differences were also studied

Heart rate had no significant positional change in either sex However, age and height of women had influence on their positional heart rate response

Systolic and mean pressure of women decreased when seated but in men only pulse pressure decreased significantly

Cardiac output and stroke volume decreased to a similar extent in both sexes when seated The systemic vascular resistance increased correspondingly Pulmonary ventilation and oxygen consumption were unchanged when seated and the arteriovenous oxygen difference was thus increased due to the decrease of cardiac output. Hematocrit increased when seated only for men

Body size had an influence on the positional response of pulmonary ventilation in men Age of women had an influence on the systemic vascular resistance response

Variances of positional responses of systolic and mean pressures, stroke volume and left ventricular work and pulmonary ventilation were higher in men than in women

IV Reproducibility of measurements at rest and during exercise

The reproducibility of hemodynamic as well as of respiratory measurements has been previously studied by Bjure (7), Brandfonbrener *et al* (9) Forsberg (16) Lund-Johansen (35), Thomasson (50) and Smith *et al* (44) The influence of age and body size on the reproducibility has not been explored The aim of the present paper is to supply further information on these problems

Subjects

The selection of the subjects has been described (part I) These subjects also took part in other studies (part II and V) Duplicate determinations at rest sitting in an armchair were made in 14 men Thirteen other men performed work loads seated on a bicycle ergometer The mean values of anthropometric data and age of the subjects are given in table I Tables of individual data are available on request

Performance

Details of the procedure have been given (part I, II and V) Polyethylene catheters were placed in the brachial artery and in the right atrium or a central vein from the antecubital fossa After a rest period of half an hour recordings were made in the seated position resting in an armchair the back of which was inclined 60 degrees from the horizontal plane The protocol included an ECG intravascular pressures collection of expired air and cardiac output (dye dilution technique) Half an hour later the procedure was repeated The exercise protocol included the same measurements The interval between the determinations was one half hour The work loads were 400 kpm/min in 6 subjects and 600 kpm/min in 7 subjects One preceding period of exercise at half the load was performed of all subjects The statistical methods as well as the methods for obtaining variables have been described (part I and V) When no difference of the two recordings was obtained the standard error of a single determination was calculated according to the formula (12)

$\sqrt{\sum \frac{(x_2-x_1)^2}{2n}}$, where x_1 and x_2 are the two determinations of each subject and (n) number of subjects

Results

Mean values and differences are given in tables II and III with standard deviations and standard errors of a single determination Regression equations using the first determination as independent variable to predict the repeated determination as dependent variable are given in table IV and V Simple regressions with age and anthropometric measurement as independent variable (x) and the difference between the repeated and first determination as dependent variable (y) are given in table VI

Heart rate (HR)

Individual values are given in fig 1 and 2

Table I *Age and anthropometric data in men where duplicate determinations of circulatory variables were made Mean values ($\bar{x}$) standard deviations (s_x) number of subjects (n)*

	Rest n = 14		Exercise n = 13	
	$\bar{x}$	s_x	$\bar{x}$	s_x
Age (years	36 9	11.5	40 0	11 0
Height (m)	1 756	0 044	1 761	0 056
Weight (kg)	71 2	10 4	72 0	11.3
Body surface area (m)	1 861	0 130	1 878	0 149

Table II *Mean values ($\bar{x}$) and standard deviations (s_x) of duplicate determinations (x_1 and x_2) of circulatory and respiratory variables sitting at rest in 14 men t value of difference (t) probability level (P) standard error of a single determination $\left(\sum\frac{(x-x_1)}{2n}\right)$ For abbreviations see text*

Variable	$\bar{x}_1$	s_{x_1}	$\bar{x}_2$	s_x	$\overline{x_2-x_2}$	$s_{x_2-x_1}$	t	P	$\sum\frac{(x_2-x_1)^2}{2n}$
HR beats/min	65.2	8.3	61 7	8 0	−3 5	3 9	3.33	<0 01	
Ps_{BA} mm Hg	128 7	10 0	127 1	8 6	−1 6	3 9	1.51		2 88
PD_{BA} mm Hg	74 1	8 7	74 1	8 0	0 0	2 4	0		1 63
$\bar{P}_{BA}$ mm Hg	94 7	9 4	93 6	8.2	−1 1	3 4	1 18		2.43
Ps_{BA} PD_{BA} mm Hg	54 6	5 9	53 0	6.3	−1 6	3.3	1 78		2.51
Q l/min	5 96	1 07	5 78	1 10	−0 18	0 59	1 18		0 423
SV ml	91 6	13 0	93 9	14 4	2 2	8 0	1 03		5 70
LVW kpm/min	7 73	1 77	7.35	1 44	−0.39	0 91	1 59		0 678
LVSW pm	119	23	119	18	1	13	0 21		8 69
SVR mm Hg/l/ min	16.3	2.6	16 7	3 1	0 4	1 4	1 15		1 00
SVC ml/min/mm Hg	63 1	10 7	62 4	13 7	−0 8	5 9	0 50		4 05
VO_2 ml(STPD)/min	259 8	30 8	260.3	32 5	0 5	33 9	0 06		23 10
V_E l (BTPS)/min	7 60	1 40	7 51	1.34	−0 09	0 87	0 40		0 597
R	0 791	0 048	0 764	0 026	−0 026	0 053	1 87	(<0 10)	0 0407
$(a-\bar{v})O_2$ ml/l	44.3	5 7	45 9	7 4	1 6	5 9	1 17		3 60
Hct	42.1	2.3	42 4	2.4	0.3	0 8	1 30		0 598

Table III *Mean values ($\overline{x}$) and standard deviations (s_x) of duplicate determinations (x_1 and x) of circulatory and respiratory variables during exercise in 13 men For abbreviations see table II and text*

Variable	$\overline{x}_1$	s_{x_1}	$\overline{x}$	s_x	$\overline{x - x_1}$	$s_{x_2 - x_1}$	t	P	$\sum \frac{(x - x_1)^2}{2n}$
HR beats/min	135 5	22 8	140 7	25 9	5.2	5.2	3.58	<0 005	—
$P_{S_{BA}}$ mm Hg	168 6	18 9	166 8	17 4	− 1 8	5 0	1.33		3 64
$P_{D_{BA}}$ mm Hg	85 7	7.2	86 1	7 9	0 4	2 9	0 47		2.01
$\bar{P}_{BA}$ mm Hg	116 3	9.3	115 1	8 3	− 1 2	4 0	1 12		2.83
$P_{S_{BA}}$ $P_{D_{BA}}$ mm Hg	82 9	17 0	80 7	15 9	− 2.2	5 3	1 51		3 94
Q l/min	13 09	2 33	12 80	1 92	− 0.29	0 92	1 15		0 656
SV ml	97 8	17.2	92 5	15.2	− 5.2	6 7	2 82	<0 02	
LVW kpm/min	20 70	4 51	20 09	3 62	− 0 61	1 94	1 13		1.39
LVSW pm	156	33	146	28	−10	14	2 52	<0 05	
SVR mm Hg/l/min	9 15	1 67	9 17	1.39	0 02	0 59	0 14		0 402
SVC ml/min/mm Hg	112 8	20 6	111 2	16.3	− 1 6	7 7	0 76		5.33
V_O ml(STPD)/min	1477	260	1530	293	53	59	3.26	<0 01	
V_E l (BTPS)/min	41 08	12 03	41 96	12 72	0 88	2.30	1.39		1 68
R	0 932	0 061	0 908	0 051	− 0 025	0 021	4 13	<0 005	
$(a-\bar{v})O$ ml/l	113 6	13.3	119 6	13 5	6 0	8 4	2.59	<0 025	
Hct	45 4	2 8	45.2	2 4	− 0.2	1 0	0 56		0 679

Table IV *Duplicate determinations of hemodynamic and respiratory variables in 14 men sitting at rest The first determination was used as independent variable (x) and the second determination as dependent variable (y) in linear regression Residual standard deviation (s_{yx}) t value of regression coefficient (t) correlation coefficient (r) For abbreviations see text*

Eq nr	y	Regression equation	s_{yx}	t	P	r
1	HR	y = 6.3 +0 850 x	3 9	6 53	<0 001	0 883
2	$P_{S_{BA}}$	y = 25 1 +0 793 x	3 4	8.36	<0 001	0 923
3	$P_{D_{BA}}$	y = 8 0 +0 892 x	2.3	12.25	<0 001	0 962
4	$\bar{P}_{BA}$	y = 16 4 +0 815 x	3 0	9.22	<0 001	0 935
5	$P_{S_{BA}}-P_{D_{BA}}$	y = 2 9 +0 917 x	3 4	5 77	<0 001	0 857
6	Q	y = 0.56 +0 875 x	0 60	5 68	<0 001	0 853
7	SV	y = 9 2 +0 924 x	8.3	5.23	<0 001	0 834
8	LVW	y = 1 96 +0 697 x	0 77	5 80	<0 001	0 858
9	LVSW	y = 40 4 +0 666 x	10 6	5 16	<0 001	0 830
10	SVR	y = −0 72 +1 07 x	1 44	7 00	<0 001	0 896
11	SVC	y = −12 0 +1 18 x	5 8	7 84	<0 01	0 914
12	V_O	y = 142 8 +0 452 x	30 6	1 64	(<0 20)	0 429
13	V_E	y = 1 68 +0 766 x	0 84	4 59	<0 001	0 798
14	R	y = 0 739+0 032 x	0 027	0.21	(<0 90)	0 059
15	$(a-\bar{v})O$	y = 3.3 +0 962 x	5.2	3 80	<0 005	0 739
16	Hct	y = 0 7 +0 991 x	0 86	9 48	<0 001	0 939

Table V *Duplicate determinations of hemodynamic and respiratory variables during bicycle exercise in 13 men. The first determination was used as independent variable (x) and the second as dependent variable (y) in linear regression. For abbreviations see table IV*

Eq nr	y	Regression equation	s_{yx}	t	P	r
17	HR	y = −11 0 +1 12 x	4 6	19 5	<0 001	0 985
18	P_{SBA}	y = 16 1 +0 893 x	4 7	12 4	<0 001	0 966
19	P_{DBA}	y = − 1 1 +1 02 x	3 1	8.28	<0 001	0 928
20	$\bar{P}_{BA}$	y = 21 5 +0 804 x	3 7	7 10	<0 001	0 906
21	$P_{SBA}-P_{DBA}$	y = 6 7 +0 893 x	5.2	10 2	<0 001	0 950
22	Q	y = 2 83 +0 761 x	0 76	8 11	<0 001	0 925
23	SV	y = 13 0 +0 813 x	6 1	7 93	<0 001	0 922
24	LVW	y = 4 99 +0 730 x	1 58	7 26	<0 001	0 909
25	LVSW	y = 27.3 +0 761 x	12 2	7 15	<0 001	0 907
26	SVR	y = 2 00 +0 784 x	0 49	9 24	<0 001	0 941
27	SVC	y = 27.3 +0 744 x	5 8	9 16	<0 001	0 940
28	V_O	y = − 106 +1 11 x	54	18 7	<0 001	0 984
29	V_E	y = 0 078+1 04 x	2 33	18 7	<0 001	0 984
30	R	y = 0 183+0 777 x	0 017	9 65	<0 001	0 945
31	$(a-\bar{v})O$	y = 26 9 +0 816 x	8 4	4 50	<0 005	0 804
32	Hct	y = 8 7 +0 804 x	0 9	9 05	<0 001	0 938

Table VI *Regression analyses of differences between repeated measurements of hemodynamic and respiratory variables at rest (R) (n = 14) and during exercise (E) (n = 13) used as dependent variables (y) and age or anthropometric measurements used as independent variables (x). For abbreviations see text and table I and IV*

Eq nr	y	x	Regression equation	s_{yx}	t	P	r
33	$(HR - HR_1)$ R	BSA	y = 16 71 x− 34 61	3 39	2 34	<0 05	0 557
34	$(HR - HR_1)$ E	age	y = −0 286 x+ 16 6	4.31	2 53	<0 05	−0 607
35	$(V_O - V_{O_1})$ R	age	y = −1 66 x+ 61 8	28 9	2 38	<0 05	−0 563
36	$(V_O - V_{O_1})$ E	weight	y = −3 13 x+278 9	49.3	2 50	<0 05	−0 601
37	$(V_O - V_{O_1})$ E	BSA	y = −220 1 x+466 6	51 2	2 23	<0 05	−0 558
38	$(V_E - V_{E_1})$ R	age	y = −0 0503 x+ 1 765	0 675	3 48	<0 01	−0 666

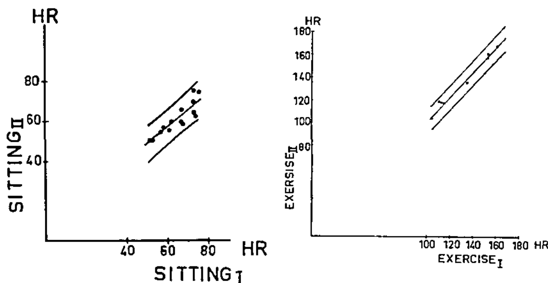


Fig 1 and 2 Regression lines and 95 % confidence limits for the dependence of the repeated determination (y) of heart rate HR (beats/min) on the first determination (x) at rest (1) and during exercise (2)

At rest, the repeated heart rate was lower and a regression on body surface area was found for this difference (33) The repeated determination of heart rate was comparably lower for those having a smaller surface area than those with a bigger surface area The increase of precision due to regression was 115 % (standard deviation of difference before regression over residual standard deviation of regression)

During the repeated period of exercise the heart rate was higher and a regression on age was obtained for this difference that indicated a higher difference in young subjects (34)* No body size dependence was found The precision increased to 121 % when regression was used The correlation coefficient between the repeated determinations of heart rate during exercise (17) was higher than the correlation coefficient between the corresponding measurement during rest (1)* ($P<0.02$) No significant deviations from the normal distribution of skewness (g_1) or kurtosis (g_2) were found for heart rates

Brachial arterial pressure Brachial arterial systolic ($P_{S_{BA}}$) diastolic ($P_{D_{BA}}$) mean ($\bar{P}_{BA}$) and pulse pressure ($P_{S_{BA}} - P_{D_{BA}}$)

Individual values are given in fig 3 and 4 of the mean pressures

At rest the repeated pressure recordings were not significantly different from the first and no regression on age or body size was found For the repeated period of exercise no differences were

*) Eq nr according to table IV V or VI

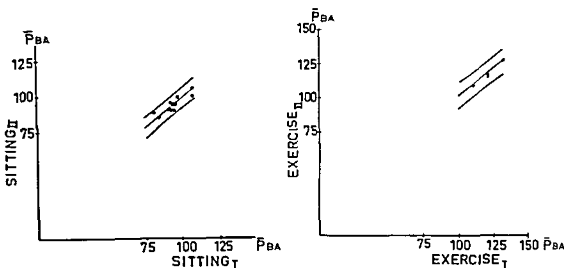


Fig 3 and 4 Regression lines and 95 % confidence of the repeated determination (y) of brachial arterial mean pressure P̄BA (mm Hg) on the first determination (x) at rest (3) and during exercise (4)

found either No regression on age or body size was found for the differences of the two determinations during exercise No differences between the corresponding correlation coefficients of the duplicate determinations during rest and exercise were found (2—5)* and (18—21)* No significant deviations from the normal distribution of g_1 and g_2 were obtained for pressures

Flow-dependent variables Cardiac output (Q), stroke volume (SV) left ventricular work (LVW) and stroke work (LVSW) systemic vascular resistance (SVR) and conductance (SVC)

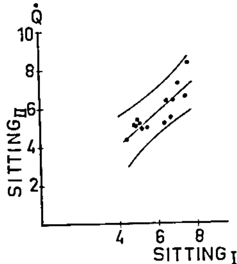
The individual values are given in fig 5—10

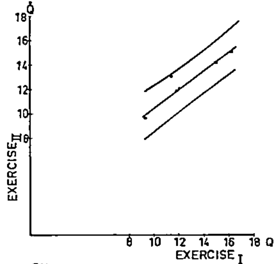
The repeated determinations at rest were not different No regression on age or body size was found for the differences of the two determinations During the repeated exercise, however, the stroke volume (23)* (fig 8) and the calculated stroke work (25) were significantly smaller No regression on age or body size was obtained during exercise The corresponding correlation coefficients during rest and exercise were not different (6—11)*, (22—27)* No deviations from the normal distribution of g_1 and g_2 were found

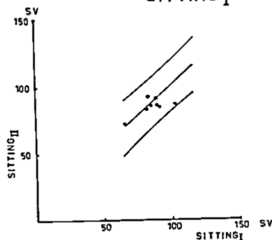
Respiratory variables Oxygen consumption (V_{O_2}) pulmonary ventilation (V_E) ventilatory exchange ratio (R) and arteriovenous oxygen difference ($(a-\bar{v})_{O_2}$)

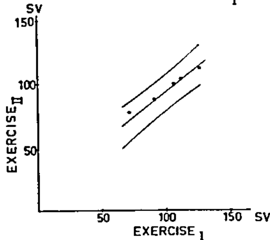
Individual values are given in fig 11—13

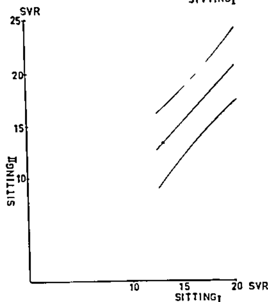
At rest no differences were found for the mean values of these measurements Regressions on age were found of the differences of pulmonary ventilation

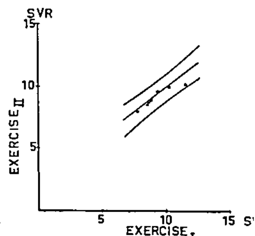

Q̇
10
8
6
4
2
SITTING II
4
6
8
SITTING I


Q̇
18
16
14
12
10
8
EXERCISE II
Q̇
8
10
12
14
16
18 Q
EXERCISE I


SV
150
100
50
SITTING II
50
100
150 SV
SITTING I


SV
150
100
50
EXERCISE II
50
100
150 SV
EXERCISE I


SVR
25
20
15
10
SITTING II
10
15
20 SVR
SITTING I


SVR
15
10
5
EXERCISE II
5
10
15
EXERCISE I

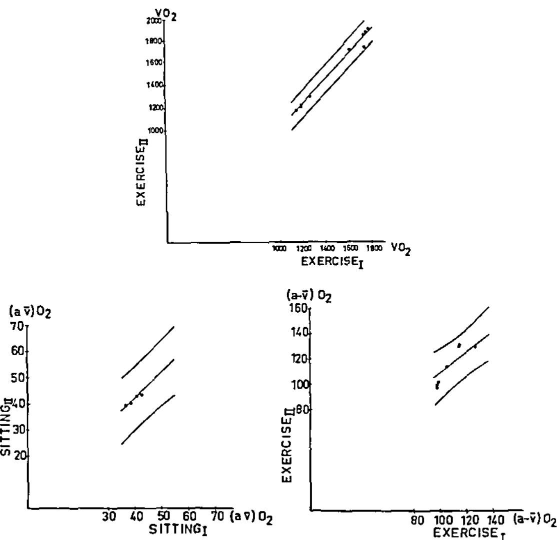


Fig 11 12 and 13 Regression lines and 95 % confidence limits of the dependence of the repeated determination (y) of oxygen consumption V_O (ml/min) on the first determination (x) during exercise (11) and the corresponding figures for arteriovenous oxygen difference (a–v̄)O (ml/l) at rest (12) and during exercise (13)

(38) and oxygen consumption (35) The younger men had comparably higher oxygen consumption and pulmonary ventilation on the repeated determination

During the repeated exercise, the oxygen consumption was significantly high er but not the pulmonary ventilation The arteriovenous oxygen difference was also higher on the repeated exercise The

ventilatory exchange ratio was lower The reproducibility of the oxygen consumption estimated from the differences between the two periods of exercise, had regressions on each of weight (36) and body surface area (37) Those with smaller body size had comparably higher oxygen consumption at the repeated work load The increases of precision due to regression were 120 and 115 % respectively, for weight and body surface area

The coefficients of correlation for the repeated determinations of oxygen consumption and ventilatory exchange ratio at rest did not reach significant levels (12, 14) and were significantly lower than the corresponding correlation coefficients during exercise (28 30) ($P<0\ 01$ and $P<0\ 01$ respectively) The correlation coefficients between the repeated determinations of pulmonary ventilation and arteriovenous oxygen difference were significant at rest (13, 15) and during exercise (29, 31) The correlation coefficient between the repeated pulmonary ventilations during exercise was higher than the corresponding coefficient at rest ($P<0\ 01$)

Deviations from the normal distribution of g_1 and g_2 were obtained only for *ventilatory exchange ratio during the* first determination at rest ($g_1 - 1\ 62$, $P<0\ 02$ and $g_2\ -3\ 41$ $P<0\ 02$)

Hematocrit (Hct)

No changes of hematocrit were obtained

The variances of the hemodynamic and respiratory variables during the first and repeated recordings were not significantly different at rest or during exercise

Discussion

The target population of circulatory healthy adult men is hard to define due to the non random selection of subjects However for the present purpose of analysing the reproducibility this factor was probably of minor importance There were no skewness or kurtosis of age or anthropometric data There were no significant correlations between age and body size The only deviations found from normality of the hemodynamic or respiratory data were the slight positive skewness and kurtosis of ventilatory exchange ratio Thus non normality of the distributions or correlations between the independent variables did not invalidate the present regression analysis

At rest the reproducibility was good The decreased heart rate at the repeated determination was similar with the decrease of repeated heart rate laying at rest described by Bjure (7) Decreases of cardiac output and stroke volume and a tendency to lower oxygen consumption were also obtained in that study That decrease of oxygen consumption was probably influenced by the higher mean age of the subjects The age corrected value according to the present regression equation (35) was 11 1 ml/min of oxygen consumption decrease Good agree

ment of rest cardiac output determinations at similar interval has been reported (44) At shorter interval, 12 min, a lower standard error of a single determination for cardiac output, 4 2 % of the mean value compared to the present figure 7 4 % has been reported by Lund-Johansen (35) At an interval of 20 minutes in the supine position Lee *et al* (34) arrived at a similar figure 7 8 %

A ' steady state at rest is hard define The first determination may result in more apprehension than a later determination The duration of the rest periods may influence However, these kind of influences should cause a change in variability between rest and exercise determinations which was not always evident in this study Age and body size may also have an influence on the ' steady state' as seen from the regressions Those with a large body surface area had no difference in heart rate when measurements were repeated Age also had an influence on the steady state The younger subjects had comparably elevated ventilations and oxygen consumptions when measurements were repeated

The standard error of a single determination for oxygen consumption in the sitting position seemed to be somewhat higher than in recumbent position Thomasson (50) has reported 14 89 ml/min or 6 2 % of the mean value compared with 23 1 ml/min or 8 9 % in the present of study ($P < 0\ 05$)

During exercise the reproducibility was not as good as at rest, when mean values were considered The correlations between the repeated measurements of heart rate, oxygen consumption, pulmonary ventilation, and ventilatory exchange ratio during exercise were higher than the corresponding correlations at rest The variability was comparably smaller for the determinations during exercise The response of the repeated heart ra e during exercise was opposite to that at rest At a repeated smaller work *load in the supine position*, an indicative increase of heart rate, a decrease of arterial mean pressure, an unchanged cardiac output and a smaller stroke volume have been reported (7) In another study of normal subjects no changes were obtained of these variables (59) A smaller standard error of a single determination of cardiac output repeated at 2 minutes interval has been reported previously by Lund-Johansen (35) It was 3 6 % of the mean value compared to the present figure 5 1 %

Of the respiratory variables, the oxygen consumption was higher but the pulmonary ventilation was the same when the work was repeated The decrease of ventilatory exchange ratio might explain this More glucose and less fat are consumed in the metabolism when the ratio is high Relatively less oxygen is also needed to furnish the same amount of energy The available amount of muscle glycogen for utilization might have been higher for the preceding exercise as indicated by the investigations of Hermanson *et al* (26) Proportionally more fatty acid, which needs more oxygen for each energy unit delivered was thought to have been utilized during the repeated exercise This mechanism does not work when an oxygen debt is built

up, but the present submaximal loads do *not cause any oxygen debt (57)*

The age dependence of the reaction of oxygen consumption at repeated periods of exercise may be due to a more constant ratio of glucose/fat for combustion in the older men In the heavier and larger men the same explanation *might be proposed*

The high coefficients of correlation especially during exercise between the duplicate determinations indicated a high degree of validity and precision of the methods used

Summary

Duplicate hemodynamic and respiratory determinations at half hour intervals were made in 14 men at rest and in 13 men during exercise

Reproducibility was good at rest when *determined from the mean* values and correlation coefficients Only the mean value of heart rate was different being lower for the repeated measurement During exercise the reproducibility was not as good when determined from the mean values These increased for heart rate oxygen consumption and arteriovenous oxygen difference at the repeated determination The stroke volume left ventricular stroke work and ventilatory exchange ratio decreased The correlation coefficients between the repeated determinations of heart rate oxygen consumption, pulmonary ventilation and ventilatory exchange ratio were however, higher than the corresponding coefficients at rest

An influence of age on reproducibility was obtained for oxygen consumption and pulmonary ventilation at rest the *younger tending to have higher* values when the measurements were repeated Age influence during exercise was found for heart rate the younger subjects having a comparably higher heart rate for the repeated exercise

Body surface area dependence was found for heart rate at rest The heart rates were comparably lower in smaller men at the repeated determination The oxygen consumption during exercise was comparably lower at the repeated exercise in men with larger body surface area Weight had a similar influence on the exercise oxygen consumption

V Studies during exercise sitting on a bicycle ergometer

by G Schroder, R Malmcrona & R Sannerstedt

Exercise studies have been used extensively to evaluate the circulatory and respiratory adaptation in health and disease Many kinds of exercise have been used e g step test walking or running on a treadmill bicycle exercise in recumbent or sitting position Much knowledge of the circulation in health and disease has thus been collected The present study was made to supply more information about the influence of age and body size on the circulation in men and women Sex differences of the circulatory and respiratory variables were tested before and after adjustments according to the regressions obtained on body size and age to see if they could explain the sex differences

Subjects

Fifty nine men and twenty eight women took part in the study* The selection of the subjects has been described and a detailed presentation of their distributions of age and body size has been given (part II)

Performance

Details have been given (part I) In short a polyethylene catheter was introduced from the antecubital vein to a central vein or to the right atrium A second catheter was placed in the brachial or axillary artery 20—30 cm proximal to the antecubital fossa

The subjects had fasted at least 12 hours before the procedure which began in the morning in a quiet room One set of measurements included heart rate intraarterial pressures, cardiac output by a dye dilution technique and collection of expired air to analyse pulmonary ventilation, oxygen consumption and ventilatory exchange ratio Hematocrit was also determined One set of measurements was made at rest in the sitting position half an hour before the period of exercise (part II) In some a previous set of measurements had been made in recumbent position (part I) The first period of exercise was followed by a second one in 29 men and 19 women and by a third in 22 men

The responses during the first exercise periods will be presented as the increases of the variables per unit in

*) Tables of individual data are available on request

crease of oxygen consumption and heart rate. An evaluation of the response of heart rate, oxygen consumption and cardiac output at various loads on the first, second and third period of exercise has shown significant effects of a previous exercise on the next as well as significant effects of these quotients on different loads in the same period of exercise (part VI). It was found feasible to use the increase in oxygen consumption or heart rate instead of work load to measure the amount of exercise and to express the response to exercise as the change of the variable over the increase of oxygen consumption or heart rate.

The statistical methods and other data processing details have been given for the present material (part I).

Results

The mean values, standard deviations and coefficients of variation for the changes of the variables during exercise over the increase of oxygen consumption and heart rate are given in tables I and II, respectively. The regression equations of these variables on age and body size variables are given in tables III and IV. The adjusted values to the mean age or mean body size of the other sex according to the regressions are given in table V.

Table I. *Mean values ($\bar{x}$), standard deviations ($s_{\bar{x}}$) and coefficients of variation (c. v.) of changes of hemodynamic and respiratory variables over increase of oxygen consumption (l (STPD)/min) during the first exercise period in 59 men and 28 women. For abbreviations see the text.*

	MEN			WOMEN			P value for sex differences	
	x	s_x	c. v.	x	s_x	c. v.	$\bar{x}$	c. v.
$HR/\dot{V}O_2$	49.9	11.2*)	22.4	79.3	23.3	29.4	<0.001	
$Ps_{BA}/\dot{V}O_2$	31.2	10.3*)	33.0	39.5	18.3	45.3	<0.05	
$Pd_{BA}/\dot{V}O_2$	8.25	6.77*)	82.0	9.09	11.84	130.0	–	<0.10
$P_{BA}/\dot{V}O_2$	16.5	7.0*)	42.4	21.9	13.2	60.3	<0.05	–
$Ps_{BA}-Pd_{BA}/\dot{V}O_2$	22.9	8.4*)	36.7	30.4	14.1	46.4	<0.02	
$Q/\dot{V}O_2$	6.[illegible]9	1.43*)	22.8	7.76	2.81	36.2	<0.0[illegible]	–
$SV/\dot{V}O_2$	14.7	14.1	95.9	15.8	25.7	163.0	–	<0.10
$SVR/\dot{V}O_2$	5.98	2.55*)	42.6	11.38	5.39	47.4	<0.001	
$SVC/\dot{V}O_2$	47.3	14.6*)	32.8	61.8	29.8	48.[illegible]	<0.0[illegible]5	
$\dot{V}_E/\dot{V}O_2$	[illegible]4.3	4.4	18.1	24.7	4.7	19.0	–	–
$R/\dot{V}O_2$	0.0816	0.0653*)	80.[illegible]	0.0929	0.1184	127.4	–	<0.10
$(a-\bar{v})O_2/\dot{V}O_2$	56.[illegible]	16.6*)	29.5	67.9	24.8	36.5	<0.05	
$Hct/\dot{V}O_2$	[illegible].43	1.22	50.2	3.79	1.38	36.4	<0.001	<0.05

*) different variances between men and women

Table II *Mean values* ($\bar{x}$) *standard deviations* (s_x) *and coefficients of variation* (*c.v.*) *of changes of hemodynamic an respiratory variables over increase of heart rate (per 100 beats/min) during the first exercise period in 59 me and 28 women For abbreviations see the text*

	MEN			WOMEN			P value for sex differences	
	$\bar{x}$	s_x	c.v.	$\bar{x}$	s_x	c.v.	$\bar{x}$	c.v.
$P_{S_{BA}}$/HR	65.8	28.4	43.2	50.6	22.6	44.7	<0.02	–
$P_{D_{BA}}$/HR	17.12	15.42	90.1	10.07	16.62	165.0	<0.10	<0.05
$\bar{P}_{BA}$/HR	34.8	17.9	51.4	28.4	18.7	65.8	–	–
$P_{S_{BA}}-P_{D_{BA}}$/HR	49.3	23.5	47.7	40.5	19.8	48.9	<0.10	–
Q/HR	13.17	4.03	30.6	10.29	3.76	36.5	<0.005	–
SV/HR	33.2	34.7	102.4	25.7	38.5	149.8	–	–
SVR/HR	−12.52	5.59*)	44.6	−16.01	10.15	63.5	<0.10	–
SVC/HR	98.9	37.0	37.4	82.5	40.4	47.7	<0.10	–
$\dot{V}_{O_2}$/HR	2108	479	22.7	1380	475	34.4	<0.001	–
$\dot{V}_E$/HR	50.7	12.9	25.4	34.0	12.7	37.4	<0.001	–
R/HR	0.1695	0.1355	79.9	0.1307	0.1688	129.2	–	–
$(a-\bar{v})\ O_2$/HR	119.3	48.8	40.9	97.9	58.0	59.2	<0.10	–
Hct/HR	5.02	2.46	49.0	5.03	2.07	41.1	–	–

*) different variances between men and women

Table III *Linear regression equations of changes of hemodynamic variables per l (STPD) increase of oxygen consumption during exercise (y) on age (years), height (m), weight (kg) and body surface area (m²) as independent variables (x) in 59 men (m) and 28 women (f). Residual standard deviation* (s_{yx}), *t value of regression coefficient (t), significance level (P) for regression coefficient, correlation coefficient (r). For further abbreviations see table I*

Eq nr	y	Sex	x	Regression equation	s_{yx}	t	P	r
1	HR	f	Height	$y = 496.0 - 252.0\,x$	19.4	3.56	<0.005	−0.573
2	HR	f	BSA	$y = 275.4 - 114.8\,x$	18.71	3.96	<0.001	−0.614
3	$P_{S_{BA}}$	m	Age	$y = 19.34 + 0.346\,x$	9.56	3.11	<0.005	0.386
4	$P_{S_{BA}}$ $P_{D_{BA}}$	m	Age	$y = 14.46 + 0.251\,x$	7.98	2.71	<0.01	0.338
5	SV	f	Height	$y = -341.6 + 216.2\,x$	23.4	2.55	<0.02	0.447
6	SVR	m	Age	$y = -3.969 - 0.059\,x$	2.48	2.07	<0.05	−0.264
7	SVR	m	Height	$y = -38.74 + 18.32\,x$	2.33	3.52	<0.001	0.423
8	SVR	m	BSA	$y = -15.91 + 5.132\,x$	2.43	2.54	<0.02	0.319
9	SVR	m	Age x_1	$y = -13.69 - 0.0569\,x_1$	2.37	x_1 2.06	<0.05	
			BSA x_2	$+4.97\,x_2$		x_2 2.53	<0.02	0.406
10	SVR	m	Age x_1	$y = -34.34 - 0.0413\,x_1$	2.30	x_1 1.51	<0.20	
			Height x_2	$+16.64\,x_2$		x_2 3.16	<0.005	0.460
11	SVR	f	Age	$y = -4.366 - 0.1934\,x$	4.97	2.40	<0.025	−0.424
12	SVC	f	Height x_1	$y = -401.9 + 327.5\,x_1$	28.5	x_1 2.43	<0.025	0.334
			Weight x_2	$-1.343\,x_2$		x_2 1.58	<0.20	
13	$(a-\bar{v})\ O_2$	m	Age x_1	$y = 123.4 + 0.408\,x_1$	14.7	x_1 2.38	<0.025	
			BSA x_2	$-41.8\,x_2$		x_2 3.43	<0.005	0.508

Table IV *Linear regression equations for changes of hemodynamic and respiratory variables per heart rate increase of 100/min (y) during exercise in 59 men (m) and 28 women (f) on age (years) height (m) weight (kg) and body surface area (m^2) as independent variables (x) For abbreviations see table III and text*

Eq nr	y	Sex	x	Regression equation	s_{yx}	t	P	r
14	Ps_{BA}	m	Age	y = 31 78+1 01 x	26 22	3 33	<0 005	0 402
15	$\bar{P}_{BA}$	m	Age	y = 19 42+0 4565 x	17.26	2.28	<0 05	0.288
16	$Ps_{BA}-Pd_{BA}$	m	Age	y = 21 99+0 813 x	21 81	3.20	<0 005	0.390
17	Q	f	Age	y = 5 51+0 132 x	3 48	2.33	<0 05	0 415
18	Q	f	Height	y = −58 81+41 57 x	2 759	4 16	<0 001	0 694
19	Q	f	BSA	y = −16 17+15 50 x	3 286	3 05	<0 005	0 513
20	SV	m	Age	y = 6 23+ 0 803 x	33 75	2 04	<0 05	0.261
21	SV	f	Age	y = −32.27+1 60 x	34.21	2 87	<0 01	0 490
22	SV	f	Height	y = −619 5+390.3 x	33 15	3 23	<0 005	0.535
23	SV	f	Weight	y = −122.2+2 289 x	34 00	2 94	<0 01	0 500
24	SV	f	BSA	y = −259 9+167.3 x	33 05	3 27	<0 005	0 540
25	SVR	m	Age	y = −5 79−0 200 x	5 15	3 29	<0 005	−0 404
26	SVR	m	Height	y = −72 14+33.37 x	5.30	2 82	<0 01	0.343
27	SVR	m	Age x_1	y = −54.37−0 1669 x_1	5 00	x_1 2 80	<0 01	0 475
			Height x_2	+26.57 x_2		x_2 2.32	<0 025	
28	SVR	m	Age x_1	y = −22 44−0 1954 x_1	5 02	x_1 3.34	<0 005	0 470
			BSA x_2	+8 521 x_2		x_2 2 04	<0 05	
29	SVR	f	Age	y = 2 855−0 5181 x	8 23	3 86	<0 001	−0 604
30	SVR	f	Weight	y = 22 80−0 5992 x	8 95	2 95	<0 01	−0 498
31	SVR	f	BSA	y = 49 67−38 47 x	9 1	2 72	<0 02	−0 471
32	SVC	f	Age	y = 38 4+1 216 x	38 4	2.20	<0 05	0 356
33	SVC	f	Height	y = −548 1+381 4 x	35 6	2 94	<0 01	0 500
34	SVC	f	BSA	y = −142 5+131 8 x	37 6	2 26	<0 05	0 406
35	V_{O_2}	f	Age	y = 684+19 19 x	425	2 77	<0 02	0 476
36	V_{O_2}	f	Weight	y = −1037+37 4 x	363	4.50	<0 001	0 661
37	V_{O_2}	f	BSA	y = −2951+2536 x	362	4.53	<0 001	0 663
38	V_E	m	Age x_1	y = −52 80+0.306 x_1	12 69	x_1 2 03	<0 05	0.248
			Height x_2	+52 18 x_2		x_2 1 80	<0 10	
39	V_E	f	Weight	y = −14 79+0 755 x	11.23	2.94	<0 01	0.500
40	V_E	f	BSA	y = −53 84+51 44 x	11.20	2.97	<0 01	0 503
41	$(a-\bar{v})O$	m	Age	y = 65 61+1.58 x	45 4	2.99	<0 005	0.375
42	$(a-\bar{v})O$	f	Weight	y = −134 17+3.59 x	50 44	3 11	<0 005	0 521
43	$(a-\bar{v})O_2$	f	BSA	y = −267 76+214 2 x	52 49	2 63	<0 02	0 459
44	Hct	f	BSA	y = −7 89+7.57 x	1 875	2 61	<0 02	0 455

Table V *Adjusted mean values ($\bar{Y}_x$) and residual standard deviations (s_{yx}) for the mean values of age and body size of the other sex. Comparisons with the other sex were made. If regressions were found for that sex the lowest residual standard deviation was used. For other abbreviations see the text.*

Eq nr	Variable	Sex	Adjustment for	$\bar{Y}_x$	s_{yx}	Sex difference of mean values p
1	Δ HR/Δ VO_2	f	Height	45.28	21.93	–
2	„	f	BSA	53.24	20.15	–
3	Δ P_{SBA}/Δ VO_2	m	Age	32.13	9.65	<(0.1)
4	Δ ($P_{SBA}-P_{DBA}$)/Δ VO	m	Age	23.58	8.05	<0.025
5	Δ SV/Δ VO_2	f	Height	44.99	26.42	<0.01
6	Δ SVR/Δ VO_2	m	Age	−6.14	2.50	<0.001
7	„	m	Height	−8.45	2.45	<0.01
8	„	m	BSA	−7.14	2.49	<0.001
9	„	m	Age+BSA	−7.26	2.43	<0.001
10	„	m	Age+Height	−8.34	2.43	<0.01
11	„	f	Age	−10.86	5.06	<0.001
12	Δ SVC/Δ VO_2	f	Height+Weight	82.5	35.5	<0.001
13	Δ $(a-\bar{v})$ O/Δ VO_2	m	Age+BSA	66.8	15.1	–
14	Δ P_{SBA}/Δ HP	m	Age	68.5	26.5	<0.005
15	Δ $\bar{P}_{BA}$/Δ HR	m	Age	36.0	17.4	<(0.1)
16	Δ ($P_{SBA}-P_{DBA}$)/Δ HR	m	Age	51.5	22.0	<0.05
17	Δ Q/Δ HR	f	Age	9.95	3.55	<0.001
18	„	f	Height	15.90	3.11	<0.005
19	„	f	BSA	13.81	3.51	–
20	Δ SV/Δ HR	m	Age	35.37	34.05	–
21	„	f	Age	21.49	34.84	–
22	„	f	Height	78.39	37.47	<0.001
23	„	f	Weight	51.31	35.67	<0.05
24	„	f	BSA	63.68	35.58	<0.001
25	Δ SVR/Δ HR	m	Age	−13.06	5.20	<(0.1)
26	„	m	Height	−17.02	5.58	–
27	„	m	Age+Height	−16.56	5.28	–
28	„	m	Age+BSA	−14.98	5.15	–
29	„	f	Age	−14.61	8.38	–
30	„	f	Weight	−22.62	9.39	<0.001
31	„	f	BSA	−24.74	9.80	<0.001
32	Δ SVC/Δ HR	f	Age	79.3	39.1	<0.05
33	„	f	Height	134.0	40.2	<0.001
34	„	f	BSA	112.4	40.5	–
35	Δ VO_2/Δ HR	f	Age	1328	433	<0.001
36	„	f	Weight	1799	381	<0.005
37	„	f	BSA	1956	390	–
38	Δ V_E/Δ HR	m	Age+Height	44.5	13.39	<0.001
39	„	f	Weight	42.46	11.78	<0.01
40	„	f	BSA	45.68	12.06	<(0.1)
41	Δ $(a-\bar{v})$ O_2/Δ HR	m	Age	123.6	45.8	<0.025
42	„	f	Weight	138.1	52.9	<(0.10)
43	„	f	BSA	146.5	56.5	<0.025
44	Δ Hct/Δ HR	f	BSA	6.75	2.02	<0.005

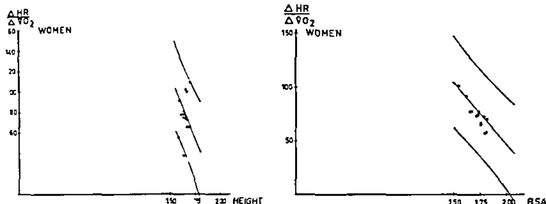


Fig 1 2 Regress on l nes and 95 % conf dencel m ts for the dependence of the change of heart rate (beats m n) over the change of oxygen consumpt on (l m n) dur ng exerc se $(\frac{\Delta HR}{\Delta VO})$ on he ght *(m) (1) and body surface area (m) (BSA) (2) n women*

Heart rate (HR beats/mın)

The heart rate increase over the oxygen consumption increase was higher in women than in men and the standard deviation was also higher The coefficients of variation were not different

No regression on age was found For women regressions on body dimensions were found (1 2) fig (1 2) After adjustments for differences in height and body surface area sex differences were not significant (table V) The standard deviations however remained higher in the group of women The increases in precision due to regression 1 d the non regress on standard deviation over the residual standard deviation were 120 *and 125 % respectively for the regressions on height and body surface area*

Deviations from the normal distribution of skewness (g) or kurtosis (g_2) were not found for heart rate increase over oxygen consumption increase in men or women

Brachial arterial pressures Increase of pressures over increases in oxygen consumption and heart rate for brachial arterial systolic ($P_{S_{BA}}$), diastolic ($P_{D_{BA}}$) mean (P_{BA}) and pulse pressures ($P_{S_{BA}} - P_{D_{BA}}$) in mm Hg

The systolic pressure increased more in women than in men per unit of oxygen consumption increase but less when the systolic pressure increase over heart rate increase was considered The same type of change was seen for the mean pressure and pulse pressure responses per unit oxygen consumption increase but not per unit heart rate increase Only the diastolic pressure response over heart *rate increase had an indicative (0 10 >* P > 0 05) sex difference Thus a tendency to a smaller pressure rise over heart rate rise of women compared to

*) Equat on number accord ng to tables III—V

men was found No significant differences between the coefficients of variation of the pressure changes over oxygen consumption increase and the same pressure changes over the heart rate increase were found within the sexes Between the sexes, the only difference of the corresponding coefficients of variation was a higher value for women of diastolic pressure change per heart rate increase The standard deviations of the pressure changes over oxygen consumption increase were all significantly higher in women than in men, but the pressure changes over heart rate increase had no sex difference for the standard deviation

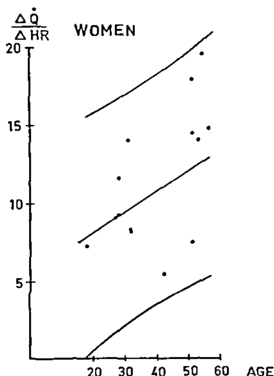
No regression on body size was found in either sex For men regressions on age were found for the systolic pressure increase (3)* and pulse pressure increase (4)* over oxygen consumption increase The age-adjusted systolic pressure response was only indicatively different from the corresponding value in women, but for the pulse pressure change the sex difference remained after adjustment The sex differences in standard deviations remained after adjustment The gains in precision due to regressions were slight, 108 and 105 % respectively

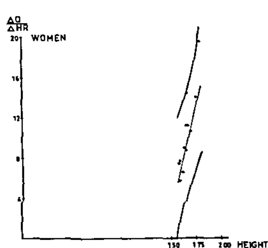
For the pressure changes over heart rate increase, dependences on age were obtained also for the systolic (14)*, mean (15)* and pulse pressure (16)* response in men The age-adjusted values for systolic and pulse pressure changes over increase of heart rate were higher than the corresponding values for women, but for the mean pressure reaction only an indicative change was found The standard deviations were not significantly different

There were several deviations from the normal distribution of pressure changes over oxygen consumption increase and heart rate increase The systolic pressure change over oxygen consumption increase had significant skewness, and kurtosis ($g_1 = 1\,43$, $g_2 = 4\,74$, $P<0\,001$ for both) in men, but not in women The systolic and diastolic pressure changes over heart rate changes in men also had significant skewness and kurtosis, ($g_1=2\,13$ and $1\,47$, $g_2=7\,91$ and $4\,25$, respectively, $P<0\,005$ for all) The women only had significant kurtosis ($g_2=1\,91$, $P<0\,05$) of diastolic pressure change over heart rate increase The mean pressure change in men but not in women showed significant deviations For the change per oxygen consumption increase, significant skewness and kurtosis ($g_1=1\,05$, $P<0\,005$ and $g_2=2\,95$, $P<0\,001$) were found In pulse amplitude changes, the only deviation in men, was a skewness ($g_1=0\,85$, $P<0\,001$) per unit heart rate increase In women the change over oxygen consumption increase had significant skewness ($g_1 = 1\,45$, $P<0\,005$) and kurtosis ($g_2=2\,01$, $P<0\,05$) and the change over heart rate increase had significant skewness ($g_1 = 1\,18$, $P<0\,02$)

Flow variables The response of the flow dependent variables over increase of oxygen consumption and heart rate for cardiac output (Q l/min), stroke volume (SV, ml) systemic vascular resistance (SVR, mm Hg/l/min) and conductance (SVC, ml/min/mm Hg)

The increase of cardiac output over





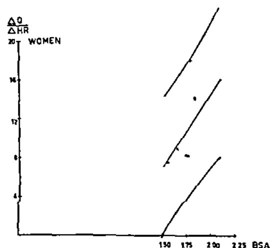


Fig 3 4 5 Regression lines and 95 % confidence limits for the dependence of cardiac output change (l/min) over heart rate change (100 beats/min) during exercise ($\frac{\Delta Q}{\Delta HR}$) on age (yrs) (3) height (m) (4) and body surface area (m) (BSA) (5) in women

increase of oxygen consumption during exercise was higher in women than in men A concomitant steeper decrease of systemic vascular resistance and increase of conductance were obtained The changes of stroke volume, however, had no sex difference The coefficient of variation of stroke volume response was indicatively lower in men than in women

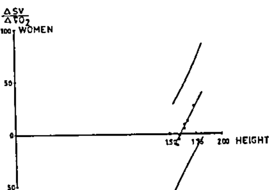
The cardiac output increase over heart rate increase was smaller in women than in men No concomitant change of stroke volume response occurred The vascular resistance and conductance responses of women were indicatively lower than in men The coefficients of variation of these variables were not different between the sexes No differences of the coefficients of variation of one of these flow dependent variables over oxygen consumption increase and the same variable over heart rate increase were found in either sex

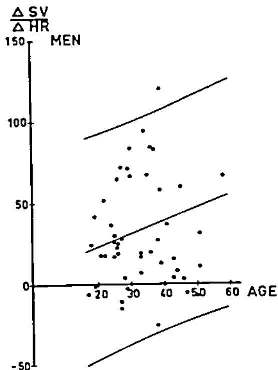
Cardiac output response to exercise had no dependence on age or body dimension in men In women the cardiac output increase over increase of heart rate had regressions on each of age (17)* (fig 3), height (18) (fig 4) and body surface area (19)* (fig 5), but no regression was found for cardiac output increase over oxygen consumption increase Adjustment for height reversed the sex difference After adjustment for body surface area no sex difference remained After age adjustment sex differences remained There were no sex differences of standard deviation for these variables, and not after regression adjustments either The increases of precision due to regression were 108, 136 and 114 %, respectively, on age, height and body surface area

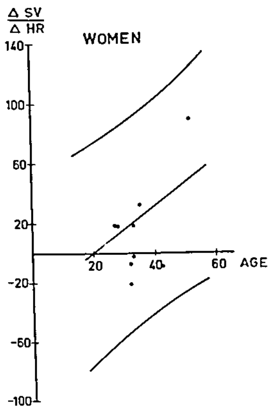
The stroke volume change per unit oxygen consumption increase had only a regression on height in women (5) (fig 6) The height adjusted value of women was higher than the mean value of men

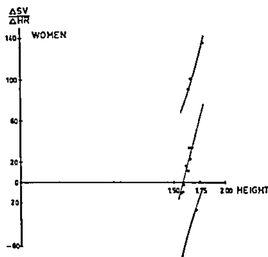
Stroke volume change per unit increase of heart rate in men had a regression on age (20) (fig 7) and in women one on each of age (21)* (fig 8), height (22)* (fig 9), weight (23)* (fig 10) and body surface area (24)* (fig 11) The adjusted values according to the regressions in women were higher than the mean value of men except for age which was not different No sex difference of standard deviation was obtained The increases of precision due to regression were 103, 113, 116, 113 and 116 %, respectively, for the regressions Identity of the regressions on age in men and women could be accepted

The systemic vascular resistance change over change of oxygen consumption in men had regressions on each of age (6) (fig 12), height (7) (fig 13) and body surface area (8)* (fig 14) and a multivariate regression on age and body surface area (9)* The regression on age and height (10)* had a low residual standard deviation but the dependence on age was not significant in that combination The adjusted values of these dependences were all higher than the mean value of women The women had only one significant regression for the resistance change over oxygen consumption change, that on age (11) (fig


ΔSV/ΔVO2
WOMEN
100
50
0
50
150
175
200 HEIGHT


ΔSV/ΔHR
MEN
150
100
50
0
-50
20
30
40
50
60 AGE


ΔSV/ΔHR
WOMEN
140
100
60
20
-20
-60
-100
20
40
60 AGE


ΔSV/ΔHR
WOMEN
140
100
60
20
0
20
-60
150
175
200 HEIGHT

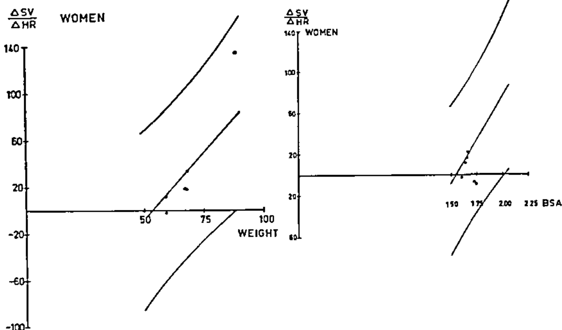


Fig 6—11 Regression lines and 95 % confidence limits for change of stroke volume (ml) over change of oxygen consumption (l/min) during exercise ($\frac{\Delta SV}{\Delta VO_2}$) on height (m) (6) in women and of stroke volume change over heart rate change (100 beats/min) during exercise ($\frac{\Delta SV}{\Delta HR}$) on age (yrs) in men (7) and women (8) and on height (m) (9) and weight (kg) (10) and body surface area (m^2) (BSA) (11) in women

15) The adjusted value was lower than the mean value of men Identity of the regressions on age in men and women was thus rejected The residual standard deviation and the level of line were different (P<0 01) The slope was indicatively different

The systemic vascular resistance change per unit increase of heart rate in women had regressions on age (29) (fig 18), weight (30)* (fig 19) and body surface area (31)* (fig 20) The age adjusted value for women was not different from the mean value of men but the mean values after adjustment for weight and body surface area were lower The sex difference of standard deviation remained in all regressions The gains in precision due to regressions were 124, 114 and 109 %, respectively, for dependence on age, weight and body surface area The resistance change over heart rate increase for men had not only a regression on age (25)* (fig 16) but also one on height (26)* (fig 17) and multiple regressions on age and height (27) as well as on age and body surface area (28)* The adjusted values for the regressions of men were not significantly different from the mean value of women However, an indicative sex difference after age adjustment was

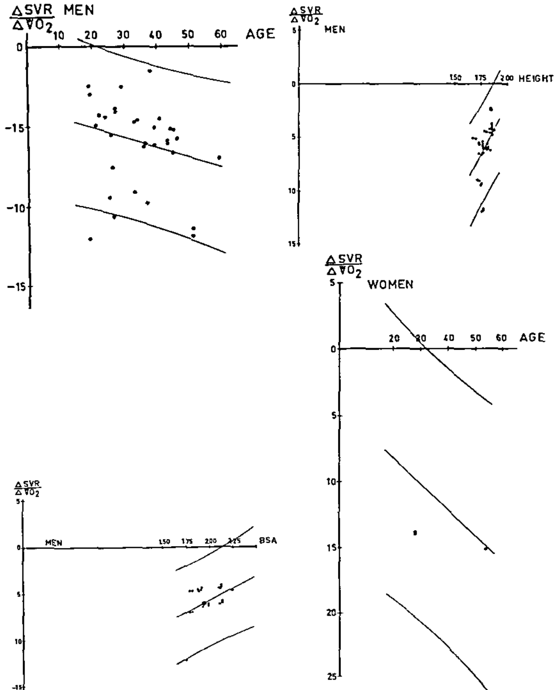
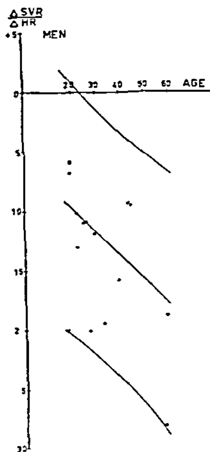
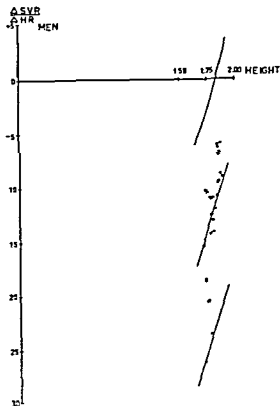


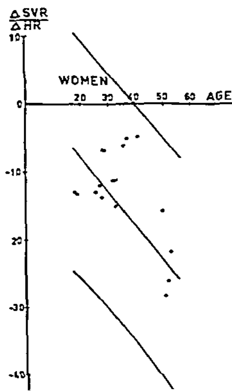
Fig 12—15 Regression lines and 95 % confidence limits for the dependence of change of systemic vascular resistance (mm Hg/l/min) over change of oxygen consumption (l/min) during exercise $(\frac{\Delta SVR}{\Delta VO})$ on age (yrs) (12) height (m) (13) and body surface area (m) (BSA) (14) in men and on age (yrs) (15) in women



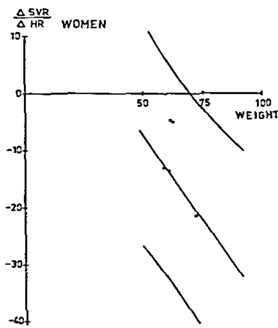
Δ SVR / Δ HR
MEN
AGE
20 30 40 50 60



Δ SVR / Δ HR
MEN
1.50 1.75 2.00 HEIGHT



Δ SVR / Δ HR
WOMEN
20 30 40 50 60 AGE



Δ SVR / Δ HR
WOMEN
50 75 100
WEIGHT

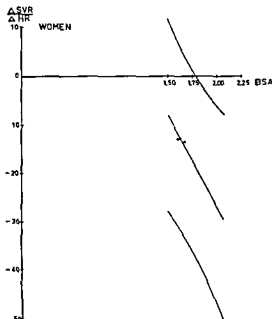


Fig 16—20 Regression lines and 95 % confidence limits for the dependence of change of systemic vascular resistance (mm Hg/l/min) over change of heart rate (100 beats/min) during exercise ($\frac{\Delta SVR}{\Delta HR}$) on age (yrs) (16) and height (m) (17) in men and on age (yrs) (18) weight (kg) (19) and body surface area (m) (BSA) (20) in women

obtained The gains in precision due to regression were 113, 105, 112, and 111 % respectively The regressions on age in men and women had no significant difference of regression coefficients but the regression lines were at different levels

For conductance change over increase in oxygen consumption no single dependence on age or body size was found When height and weight were combined as independent variables, the women had a significant dependence on height but not on weight (12)

Conductance change per unit increase of heart rate had regressions in women on age (32) height (33) and body surface area (34)* The adjusted value for height was higher than the mean value for men The standard deviations were not different The gains in precision were slight 105, 113 and 108 % respectively

The only deviation from normal distribution of cardiac output increase was found in women Their cardiac output increase over oxygen consumption increase had skewness as well as kurtosis ($g_1 = 1\ 69$, $g = 4\ 32$ $P < 0\ 001$ for both) Stroke volume change per unit oxygen consumption increase among women had significant skewness and kurtosis ($g_1 = 1\ 04$, $P < 0\ 05$ and $g = 2\ 25$ $P < 0\ 02$) The stroke volume change per

unit heart rate increase also had skewness ($g_1 = 1\ 08$, $P<0\ 025$) but not kurtosis Systemic vascular resistance change in men deviated regarding skewness for change per unit oxygen consumption increase and heart rate increase ($g_1 = -0\ 71$ and $-0\ 67$, respectively, $P<0\ 05$ for both) The women had corresponding deviations ($g_1 = -1\ 41$ and $-1\ 36$, respectively, $P<0\ 005$ for both) and the change per oxygen consumption increase also had a significant kurtosis ($g = 2\ 36$, $P<0\ 02$) The change of systemic vascular conductance had no significant deviation in men, but in women the change per unit oxygen consumption increase had skewness as well as kurtosis ($g_1 = 2\ 08$, $g_2 = 4\ 13$, $P<0\ 001$ for both)

Respiratory variables Change per unit increase of oxygen consumption and heart rate of oxygen consumption (V_{O_2}, ml (STPD)/min), pulmonary ventilation (V_E,l (BTPS)/min), ventilatory exchange ratio (R) and arteriovenous oxygen difference ($(a-\bar{v}_{O_2}$ ml/l)

Men had higher oxygen consumption and pulmonary ventilation increase per unit heart rate increase compared with women Men had an indicatively higher arteriovenous oxygen difference response per unit heart rate increase than women Ventilatory exchange ratio change over heart rate increase was not different in men and women No sex differences for the coefficients of variation or standard deviations for the change per unit heart rate increase of oxygen consumption pulmonary ventilation ventilatory exchange ratio or arteriovenous oxygen difference were found

When changes per unit oxygen consumption increase of pulmonary ventilation and ventilatory exchange ratio were observed no sex difference was detected However, the change of arteriovenous oxygen difference over increase of oxygen consumption was higher in women The response of ventilatory exchange ratio over oxygen consumption increase in men had a lower standard deviation ($P<0\ 01$) and an indicatively lower coefficient of variation than women Comparison of coefficients of variation for one variable per unit oxygen consumption increase and the same variable over heart rate increase within each sex showed only one significant change a lower coefficient in women for the pulmonary ventilation change over oxygen consumption change than the pulmonary ventilation change over heart rate change ($P<0\ 05$)

The arteriovenous oxygen difference response over oxygen consumption increase in men had a multiple regression on age and body surface area (13)* This variable had no other regression in either sex The adjusted value for age and body surface area was not different from the mean value of women The standard deviation was smaller in men also after regression adjustment The gain in precision due to regression was 113 %

Oxygen consumption increase over heart rate increase had regressions in women but not in men The regressions were on age (35) (fig 21), weight (36)* (fig 22) and body surface area (37)* (fig 23) The adjusted values for

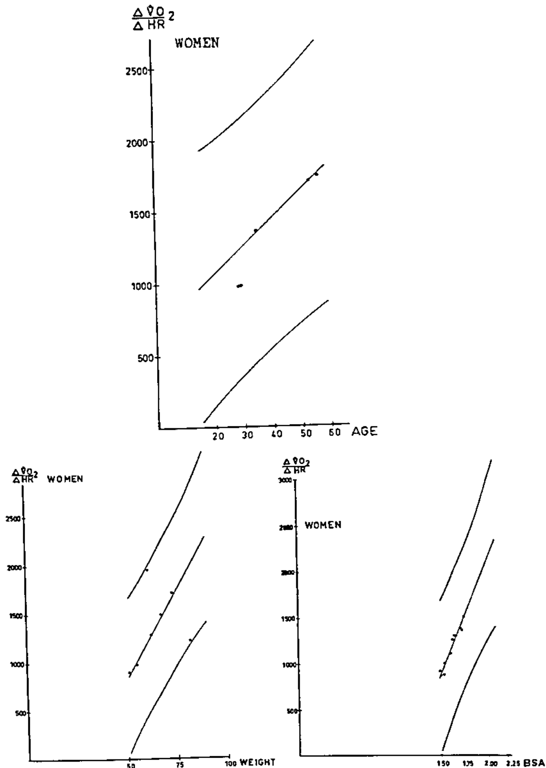


Fig 21—23 Regression lines and 95 % confidence limits for the dependence of change in oxygen consumption (ml/min) over change of heart rate (100 beats/min) during exercise $(\frac{\Delta \dot{V}O_2}{\Delta HR})$ on age (yrs) (21) weight (kg) (22) and body surface area (m) (23) in women

age and weight were lower than the mean values for men but after adjustment for body surface area no sex difference remained Standard deviations had no sex difference The gains in precision due to regressions were 112, 131 and 131 % respectively

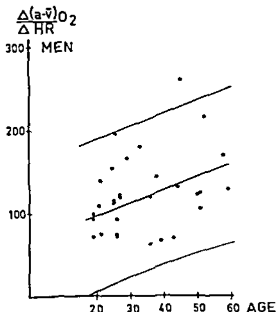
The increase of pulmonary ventilation per unit heart rate increase had a multiple regression on age and height (38)* in men The dependence on height was, however, only indicative The increase of pulmonary ventilation per unit heart rate increase in men had no single dependence on age or body size In women, however, this variable had simple regressions on each of weight (39)* and body surface area (40)* The weight adjusted value was smaller than the mean value for men but after body surface area adjustment no sex difference remained Standard deviation of this variable had no sex difference The gains in precision due to the dependence on weight and body surface area were equal, 113 %

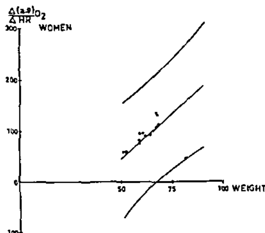
The arteriovenous oxygen difference increase per unit heart rate increase had regressions in men on age (41)* (fig 24) and in women on each of weight (42)* (fig 25) and body surface area (43)* (fig 26) The age adjusted value for men was higher than the mean value for women but weight and body surface area adjusted values of women were higher than the mean value for men Thus the group mean values had no sex difference, but the use of the three regressions revealed sex difference No sex difference of standard deviation was found The gains in precision due to these regressions were 107, 115 and 110 % respectively

The distribution in men of oxygen consumption increase over heart rate increase did not deviate from normality regarding skewness or kurtosis, but in women significant deviations were found of both ($g_1 = 1\ 65$, $g_2 = 3\ 05$, $P < 0\ 001$ for both) Pulmonary ventilation increase over heart rate increase in men had no deviation, but in women a significant skewness was found ($g_1 = 1\ 32$, $P < 0\ 01$) The pulmonary ventilation *increase over oxygen consumption increase* had significant skewness and kurtosis in men ($g_1 = 1\ 57$, $g_2 = 3\ 25$, $P < 0\ 001$ for both) as well as in women ($g_1 = 1\ 60$, $g_2 = 3\ 08$, $P < 0\ 005$ for both) The response of ventilatory exchange ratio per unit oxygen consumption increase in men had skewness ($g_1 = -0\ 76$, $P < 0\ 02$) In women this variable had kurtosis ($g_2 = 2\ 12$, $P < 0\ 025$) The response of the ventilatory exchange ratio per unit heart rate increase also had kurtosis ($g_2 = 2\ 95$, $P < 0\ 005$) Change of arteriovenous oxygen difference over oxygen consumption increase had a kurtosis in women ($g = 2\ 42$, $P < 0\ 01$) but no significant deviation in men The arteriovenous oxygen difference increase over heart rate increase had skewness in men ($g_1 = 0\ 93$, $P < 0\ 005$) and women ($g_1 = 1\ 14$, $P < 0\ 02$)

H matocrit (Hct)

The mean value of hematocrit increase over oxygen consumption increase was higher for women than for men The hematocrit increase over heart rate increase was not different in *men and*





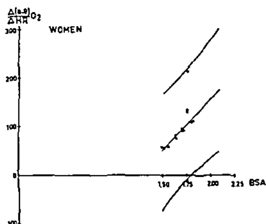


Fig 24—26 Regression lines and 95 % confidence limits for the dependence of change in arterio-venous oxygen difference (ml/l) over change of heart rate (100 beats/min) during exercise ($\frac{\Delta(a-\bar{v})O}{\Delta HR}$) on age (yrs) (24) in men and on weight (kg) (25) and body surface area (m²) (BSA) (26) in women

women The standard deviations or the coefficient of variations of the hematocrit change per unit increase of oxygen consumption or heart rate had no sex differences

One regression of hematocrit change over increase of heart rate on body surface area was obtained in women (44*) The adjusted value was significantly higher than the corresponding mean value for men In men no regression was found The gain in precision due to regression was 111 %

Distribution of hematocrit change over heart rate or oxygen consumption increase did not deviate from normality regarding skewness or kurtosis in either sex

Discussion

Methods

The effect of age, sex and body size on circulatory and respiratory variables during exercise in healthy adult subjects has been studied by several authors (1, 2 3, 5, 17, 18, 19, 23, 24, 31, 32, 35, 40, 48, 53) The combination of age and body size influence on adult men and women has not been considered in detail Age and body build influence the circulation and respiration and it is desirable especially in diagnostic work to be able to make adjustments for these variables From the physiological point of view it is also valuable to have information on these relationships Geographical differences as well as differences in physical fitness, body build and nutritional state of populations can thus be better evaluated A drawback, however, is the lack of standardization of the procedures Our results are thus not unequivocally comparable with those found in other laboratories Every laboratory needs a control material of its own and there is always need for new studies as the variables also may change with the passage of time

The non-random selection of the subjects and its consequences on the statistical evaluation have been discussed (part I) The distributions of age and body size and their deviations from normality have also been discussed (part II) The arbitrary limits of arterial pressures have contributed to skewness and kurtosis in the distribution of the pressure increase per unit oxygen consumption and heart rate increase, especially in men These deviations introduced additional uncertainty as to the inferences made from the regression equations The degree of influence is, however, limited (14) The flow variables and the respiratory variables had deviations of a smaller degree, which did not seem to invalidate the present regression analysis

Significant interrelations between the independent variables was obtained only for women Weight and body surface area had regressions on age and the method of partial correlation was used to adjust for these interrelations and so obtain the pure influence of age and body size (part II)

The method of relating the exercise to the increases of oxygen consumption and heart rate instead of to the amount of work in kpm/min has advantages (part VI). Many problems such as calibration of different ergometers, are avoided. Some important variables had no linear dependence on work load at the test levels (part VI). The loads were 200 kpm/min in seven men and 21 women, 300 kpm/min in 18 men, 400 kpm/min in one man and six women, 600 kpm/min in 30 men and one woman, 900 kpm/min in two men and 1200 kpm/min in one man. Similar calculations have been made for cardiac output and oxygen consumption to estimate deviations due to disease (25).

The increase of oxygen consumption per unit of work load has smaller variance than the increase of heart rate per unit of work load in a group of subjects but the increase of heart rate in each subject is also proportional to the work load in the present range of loads. Heart rate is easy to measure and may thus be the only alternative in many types of studies.

The quotient is easy to calculate and to compare with the present material.

Say that a woman has an oxygen consumption during the first exercise period of 1.200 l/min and at rest of 0.250 l/min. The corresponding values for heart rate are 130 and 70 beats/min. The quotient will be 130—70 over 1.200—0.250 which yields 63.2. According to the present mean value 79.3 and the standard deviation 23.3 it is evident that it is within the normal range. Information concerning weight and height makes it possible to calculate adjusted values according to eq. nr 2. Say that the calculated body surface area, which is the best independent variable, is 1.90 m^2. The adjusted mean value is 57.3 and the residual standard deviation 19.8 which is close to the observed quotient.

It is also possible to calculate a predicted mean value and standard deviation for the actual heart rate when oxygen consumption during exercise is known. The mean value (69.3) and standard deviation (11.4) in the sitting position are used as there was no regression on body size or age for women. The mean value and residual standard deviation of oxygen consumption according to regression on body surface area (eq. nr 38, part II) were 249 and 25 ml/min, respectively. The estimated increase of oxygen consumption is 1.2—0.249 = 0.95 l/min which corresponds to an increase of heart rate of 0.95 × 57.3 = 54.4, with the residual standard deviation 0.95 × 19.8 = 18.8. The predicted heart rate for that woman will be 69.3 + 54.4 = 123.7 with the standard deviation $\sqrt{11.4^2 + 18.8^2}$ = 22.0. The standard deviations of heart rate at rest and heart rate increase during exercise were considered independent. They might, however, be positively correlated, i.e. those having high heart rate at rest also have comparably high heart rates during an exercise. The given standard deviation is then too high as suggested by the large estimated 95 % confidence limits 80—168 beats/min.

The quotient was easy to use as the dependent variable (y) in regression ana-

lysis with age and body size as independent variables (x)

The use of observations from each individual at rest and during two or more periods of exercise to calculate a regression equation of the dependent variable on oxygen consumption for each one of all the subjects, and then testing the admissibility to pool the regression lines, has thus been avoided These calculations are more tedious and the expected information is probably not greater due to loss in pooling of the regression If all subjects performed the same amount of work, the calculations with quotients would have been superfluous, but the information obtained would have been confined to only the tested load Adaptation of load according to physical fitness would have been impossible and the relative work load of the subjects more varying

The results are only pertinent to the first period of exercise The quotients are often changed with successive loads (part VI)

Results

The higher heart rate increase per unit oxygen consumption increase in women was mainly due to difference in body size, since this difference disappeared when adjustment was made for body size difference according to the regression equations The correlation with body surface area in women remained also after adjustment for the age dependence of body surface area The partial correlation coefficient was —0 525 The partial correlation coefficient of age adjusted for body surface area was not significant (0 040) The lack of significant regression on body size in men was remarkable especially as the range of body size was high in men

The lack of age dependence for the heart rate increase was in accordance with some other studies (2, 6, 31) Age dependence of heart rate reaction during exercise in men but not in women has been reported The heart rate at the same level of oxygen consumption was claimed to be higher in young subjects (5) The heart rate at the same work load was also claimed to be lower in older subjects (40) A review of other studies of the relationship between heart rate during exercise and age has been given by Becklake *et al* (5) No reports of the influence of body size on heart rate response in women have been found

The brachial arterial systolic, mean and diastolic pressure increased significantly during exercise in both sexes The systolic pressure increase was more marked than the diastolic increase The pulse pressure increased correspondingly during exercise The lower rise per unit oxygen consumption increase in men for the systolic and mean pressure was partly age dependent as only an indicative sex difference remained after age adjustment of the systolic pressure increase Age dependences of this pressure response have also been reported in other studies (23, 31, 48) Per unit heart rate increase, the systolic pressure increase was higher in men than in women but the other arterial pressures were not different The age dependence in men for the systolic pressure response accentuated the sex difference and the age

dependence of pulse pressure response per unit heart rate increase added a sex difference after adjustment The age dependence of arterial pressure response may explain the lack of significant increase of systolic and mean pressure on treadmill exercise in young men reported by Tabakin *et al* (49) In younger subjects the pressure increase will be much smaller according to age adjustment

The cardiac output increase per unit oxygen consumption increase was higher in women, and the systemic vascular resistance change even more marked due to the still greater increase of mean arterial pressure per unit of oxygen consumption increase in women A smaller oxygen carrying capacity in women implied by the lower hematocrit might have contributed to the difference The significant regressions found on body size or age did not explain the sex differences of systemic vascular resistance response during exercise Differences in the vascular system probably due to another distribution of muscle tissue in relation to fat and visceral tissues might have been partly responsible rather than a different physiological response to exercise of the particular tissues in men and women The higher variance in women of these variables may be explained by a higher variability of the body composition The lack of significant regressions on body size in women of these variables also points to that mechanism The age dependence of systemic vascular resistance change per unit oxygen consumption increase remained also after adjustment for body surface area influence in women according to the partial correlation coefficient (—0 450) The partial correlation coefficient of body surface area adjusted for age was not significant (—0 193)

The higher cardiac output increase per unit oxygen consumption increase of women compared with men agreed with results from men and women 20 to 39 years of age obtained by Becklake *et al* (5)

The lack of age dependence of the cardiac output increase with increasing oxygen uptake during exercise was in accordance with some recent studies (40, 48) but not with another (5) The regression of systemic vascular resistance change on age indicating a more marked vasodilatation in the older subjects during exercise was also comparable to one study (31)

Stroke volume changes per unit oxygen consumption increase had a high variability This high variability has recently been reviewed (6) However, significant increases of mean values were found in both sexes The only regression found for stroke volume response was on height in women indicating that taller women had comparably higher increase of stroke volume per unit oxygen consumption increase than smaller The sex difference obtained after height adjustment is hard to evaluate

Of the flow dependent variables per unit increase of heart rate only the cardiac output increase had a sex difference It was higher in men and this difference was due to the body size difference In women the partial correlation coefficient of age adjusted for body surface area influence was insignificant

Difference of body surfare area but not difference of age or weight explained the sex difference of oxygen consumption and pulmonary ventilation Weight difference of women explained the sex difference of arteriovenous oxygen difference, but body surface area or age difference did not

After adjustment according to some regressions new sex differences were obtained for changes of stroke volume over oxygen consumption increase and change of pulse pressure, stroke volume, systemic vascular resistance and conductance arteriovenous oxygen consumption and hematocrit over heart rate increase

The weight and body surface area increased with age in women After partial correlation analysis, the coefficients remained significant for age and resistance change over oxygen consumption increase, age and resistance change over heart rate increase, weight as well as body surface area and oxygen consumption increase over heart rate increase weight as well as body surface area and pulmonary ventilation increase over heart rate increase, weight and arteriovenous oxygen difference change over heart rate increase The other correlations were eliminated and no new significant partial correlation coefficient was added

VI Relationships between work load and changes in oxygen consumption, heart rate and cardiac output

The purpose of this study was to obtain information concerning changes in oxygen consumption heart rate and cardiac output with increasing and successive exercise loads on a bicycle ergometer The subjects were divided into three load level groups for the first exercise period This period was made with the lowest load (200 300 or 600 kpm/min) The second exercise period was made with a higher load The third exercise period was made with the same or higher load than the second period The effect of the progression of loads as well as of the degree of load could thus be analysed

Mechanical efficiency has been shown to be lower on small exercise loads than at higher loads (2) i e more oxygen was consumed per load unit with the lower loads The mechanical efficiency was probably also age dependent (2) Thus the oxygen consumption increase per unit of time might be a better measure of the load for the subject than the quantity of load units performed per unit of time To test this hypothesis the changes of oxygen consumption heart rate and cardiac output were analysed

Change per load unit on different loads were calculated as well as change per unit oxygen consumption increase and heart rate increase Analyses of the linear regressions obtained were made to test the admissibility of using these quotients The correlations of these quotients on successive loads have also been analysed A high correlation is an indication of good precision in the method used The coefficients of variation have also been calculated to obtain a measure of the precision independent of scale changes

Subjects

The subjects presented here were selected from a larger sample presented elsewhere (part II and V) They were inpatients or paid volunteers all with a normal circulatory system

Mean values and standard deviations of age height and weight as well as the number of subjects in the different load groups are given in table I The subjects whose mean age was highest were those performing the lowest loads in the first and second exercise periods No constant differences of height or weight were found Among those who perfor-

Table I *Mean values ($\bar{x}$) and standard deviations (s_x) of age height and weight and number (n) of subjects in the groups*

Loads (kpm/min)		n	Age (yrs)		Height (m)		Weight (kg)	
			$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x
Exercise								
I	II							
200	400	7	50 7	4 1	1 76	0 06	76 6	15 0
300	600	18	29 2	8 3	1 77	0 04	74 2	15 7
600	900	4	25.3	4 9	1 83	0 02	73 6	3 3
Pooled	groups	29	33 8	12 0	1 77	0 05	74 7	14.2
Exercise								
II	III							
400	400	6	49 5	2 8	1 76	0 06	74 1	14 8
600	600	7	31 9	8.3	1 76	0 05	70 2	8 2
Pooled	groups	13	40 0	11 0	1 76	0 06	72 0	11 3
Increasing loads								
Exercise								
II	III							
Pooled	groups	9	26 9	12 6	1 77	0 04	72 3	8 4

med the second and third exercise periods on the same loads the mean age was also highest in those performing the lowest loads No differences of height or weight were obtained

Procedure

The subjects, who had been fasting for at least 12 hours were investigated in the morning One catheter was placed in the brachial artery and another in a central vein or in the right atrium from the cubital fossa (43) Determinations at rest were made while the subjects were sitting in an armchair the back of which was inclined 60° from the horizontal plane The recordings consisted of brachial arterial pressure, heart rate and cardiac output with a dye dilution technique Determinations of oxygen consumption ventilatory exchange ratio and pulmonary ventilation were made Details of these techniques are discussed elsewhere (part I)

A 30 minute rest period followed the catheterization procedure Following this rest determinations were made After another half hour of rest, the subject was seated on a bicycle ergometer and exercise started The recordings were repeated 10 minutes later with exercise continuing After another half hour of rest, a second period of exercise was performed Some subjects also performed a third period of exercise with a similar protocol

Statistical methods

Comparisons between two group mean values were made by t test and comparisons between more group mean values by analysis of variance Differences below the 5 % level were analysed according to Scheffe (10) Linear regressions were calculated according to the method of least squares and significance tests of slope and linearity were made by analyses of variance Correlation coefficients were tested after z-transformation The t test was used for comparison of 2 coefficients and χ^2-test for more than 2 coefficients Coefficients of variation were tested with a t test (60) Tests of variances were made with F-test when 2 were compared and with Bartlett s test when more variances were compared (10) Programs for calculations were written for an automatic desk computer (Programma 101, Olivetti) which was used throughout

Results

First and second periods of exercise with load increase

Twenty nine subjects performed at least two periods of exercise with increasing loads Seven had loads of 200 and 400 kpm/min, eighteen had loads of 300 and 600 kpm/min and four had loads of 600 and 900 kpm/min The results are given in table II and III and figures 1—15

The increase of oxygen consumption on successive loads paralleled the load increase from 600 to 900 kpm/min However the oxygen consumption per load unit was higher on the first load when 200 or 300 kpm/min was followed by 400 or 600 kpm/min respectively (fig 1) The variances were highest in the group that performed 300 and 600 kpm/min Two subjects in this group had comparably high oxygen consumption on both loads (1149 and 1228 ml/min on the first and 2019 and 2097ml/min respectively on the second) One of these was obese and the other anxious Oxygen consumption increase from rest per load unit was highest in the group that performed 200 kpm/min on the first period of exercise The 95 % confidence limits of the differences between this group and those on the load 600 kpm/ min and the pooled groups 300 and 600 kpm/min were 0 87 ± 0 74 and 0 62 ± 0 55 ml/kpm, respectively

During the second period of exercise at 400 600 or 900 kpm/min, however no differences of oxygen consumption per load unit were found The 95 % confidence limits for the differences between the changes from 600 to 900 kpm/min and the changes of the pooled groups from 200 and 300 kpm/ min to 400 and 600 kpm/min respectively, were 0 56 ± 0 34 ml/kpm The changes from 200 to 400 kpm/min were different from each of the changes from 300 and 600 kpm/min as well as for those groups that were pooled The 95 %

Table II *Increase of circulatory and respiratory variables during exercise for first (I) and second (II) period with increasing loads*
Symbols $\dot{V}O_2$ oxygen consumption (ml min (STPD)) HR heart rate (beats/min) Q cardiac output (l min) △ symbol of difference $\bar{x}$ mean value s_x standard deviation r coefficient of correlation c v coefficient of variation P probability n number of subjects

Loads (kpm/min)		n		△ VO I	△ VO II	△ HR I	△ HR II	△ Q I	△ Q II
I	II		$\bar{x}$	631	957	29 0	48 7	3 74	5 13
			s_x	70	65	10 2	16 9	0 71	1 28
200	400	7	r	0 881		0 952		0 848	
			c v	11 1	6 7	35 1	13 1	19 0	24 9
			$\bar{x}$	844	1478	42 4	84.3	5 17	9 17
			s_x	148	223	11.3	15 5	1 65	2 23
300	600	18	r	0 911		0 682		0 640	
			c v	17 5	15 1	26 7	18 4	31 9	24.3
			$\bar{x}$	1368	2072	62.3	96 5	10 48	14 18
			s_x	59	43	16 8	20 5	2.04	1 79
600	900	4	r	0 783		0 931		0 971	
			c v	4 3	2 1	27 0	21 5	20 0	12.6
			$\bar{x}$						
			s_x						
			r						
"Pooled groups		29	c v						

confidence limits were 0 44 ± 0 28, 0 78 ± 0 39 and 0 61 ± 0 29 ml/kpm respectively The variances for the changes from first to second period of exercise were not different

Linear regression analysis of the oxygen consumption increase for the first exercise period as the dependent variable and work load as the independent variable showed that linearity could be accepted (F_{1-6} = 0 35) The regression line intersected the y-axis significantly above the origin (fig 2) On the second period of exercise linearity could also be accepted (F_{1-6} = 1 10) and the intersection with the y-axis did not significantly deviate from the origin (fig 3)

During the first exercise periods there were lower mechanical efficiencies at smaller loads On the second loads no differences of mechanical efficiency were evident and the calculations using the quotients oxygen consumption increases per load unit could be accepted for these higher loads The lower mechanical efficiency on the first load could be explained partly by the fact that those in the lowest load group had a higher mean age Mechanical efficiency has previously been found to be low-

Table II contin

Δ VO load I	II	II I	Δ HR load I	II	II I	Δ Q 10³ load I	II	II I
3 154	2 393	−0 761	0 1450	0 1220	−0 0230	18 71	12 86	−5 86
0 350	0 159	0 224	0 0512	0 0424	0 0167	3 56	3 21	1 90
		P < 0 001			P < 0 02			P < 0 001
2 784	2 463	−0.370	0 1411	0 1405	−0 0006	17.22	15 29	−1 93
0 519	0 374	0 267	0 0376	0 0258	0 0275	5 51	3 73	4 25
		P < 0 001						
2 283	2.305	0 022	0 1035	0 1075	0 0040	17 48	15 75	−1 73
0 097	0 047	0 0(7	0 0281	0 0227	0 0109	3 39	1 96	1 58
2 804	2 475	−0 379	0 1369	0 1315	−0 0053	17 61	14 77	−2 85
0 510	0.306	0 341	0 0411	0 0317	0 0252	4 77	3 51	3 87
0 946		P < 0 001		0 827			0 849	P < 0 001
18 2	12 6		30 0	24.3		27 1	23 8	

Table III *Increase per unit increase of oxygen consumption and heart rate during first (I) and second (II) exercise period with increasing load in the pooled group (n 29) For symbols see table II*

	$\frac{\Delta Q \; 10^3}{\Delta \dot{V}O_2}$ I	II	II I	$\frac{\Delta HR}{\Delta \dot{V}O}$ I	II	II I	$\frac{\Delta Q}{\Delta HR}$ I	II	II I
$\bar{x}$	6 276	6 118	−0 158	0 0479	0 0544	0 0065	0 1339	0 1167	−0 0172
$s_{\bar{x}}$	1.393	1 435	1 258	0 0107	0 0123	0 0089	0 0365	0 0331	0 0282
r	0 604			0 708		P < 0 001	0 675		P < 0 005
c v	22 1	23 5		23 8	22 6		27.3	28.3	

er with increasing age (2) The use of the quotients oxygen consumption increase per load unit for low loads during the first period of exercise was not justified

The heart rate increase per load unit

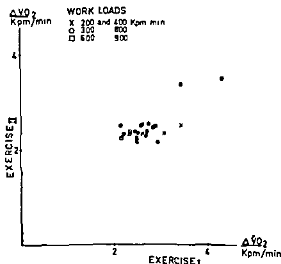


Fig 1 Diagram of increase of oxygen consumption (ml) per kpm/min ($\Delta \dot{V}O_2$/kpm/min) during the first (I) and second (II) exercise periods with 3 load groups

Fig 2 3 Linear regressions (with 95 % confidence intervals of regression mean values) for oxygen consumption increase (y) on load (x) during the first (fig 2) and second (fig 3) exercise periods Eq $y = 1\ 82x + 287$ and $y = 2\ 24x + 103$ respectively and coefficients of correlation (r) 0 877 and 0 882 Symbols as in previous fig

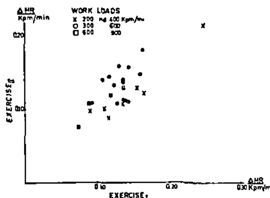


Fig 4 Diagram of increase of heart rate per kpm/min (ΔHR/kpm/min) during the first (I) and *second (II) exercise period for 3 load groups*

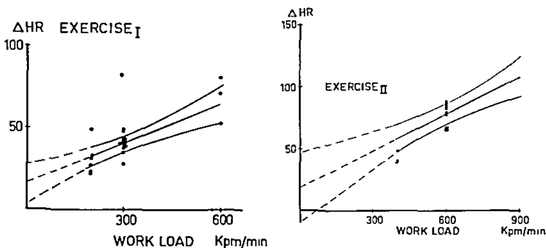


Fig 5 6 Linear regressions for heart rate increase (y) on load (x) during the first (fig 5) and second (fig 6) exercise periods Eq $y = 0.0794x + 16.7$ and $y = 0.0988x + 18.8$ respectively and r 0.640 and 0.642 Symbols as in previous fig

for the second exercise period was lower than that on the first for the 200—400 kpm/min group This difference between the first and second exercise periods was not obtained for the other two load groups (fig 4) For the first exercise *period there were no differences of heart* rate increase per load unit between the three load groups and these results were also obtained for the second period In addition, considering the changes from first to second periods there were also no significant differences between the three load groups No differences of variances were obtained

Analysis with linear regression of heart rate increase as the dependent variable and load as the independent

variable resulted in an acceptable linearity on the first exercise period ($F_{1, 6}$ = 1 21) However, the intersection with the y axis was significantly above the origin (fig 5) Similar analysis of the second period rejected the hypothesis of linearity ($F_{1, 6}$ = 6 70, P<0 05), but the intersection of the y-axis was not significantly away from the origin (fig 6)

The increase of cardiac output per load unit was higher for the first period of exercise when 200 was followed by 400 kpm/min When 300 was followed by 600 and 600 by 900 kpm/min no differences were found (fig 7) However, the increases of cardiac output from rest values per load unit for the first and second period of exercise were not different, the cardiac output increase with increasing load was not different at these submaximal loads The variances for the first period and the second period were not different The variances of the differences in cardiac output reactions between first and second exercise period were, however, different

The linear regression analysis of the first exercise period with cardiac output increase as the dependent and load as the independent variable resulted in acceptable linearity ($F_{1, 6}$ = 0 19) with an intersection that did not significantly deviate from the origin (fig 8) Similar results were obtained for the second exercise period ($F_{1, 6}$ = 0 13), (fig 9)

To evaluate the relative precision of the variables on first and second periods of exercise, the coefficients of variation of increases of oxygen consumption, heart rate and cardiac output were tested in each group as well as in the pooled groups No significant differences were found

To evaluate the correlation between the variables for the first and second exercise periods in the three load groups and to test the correlations of the variables studied within each load groups,

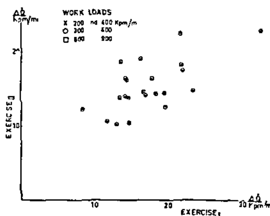
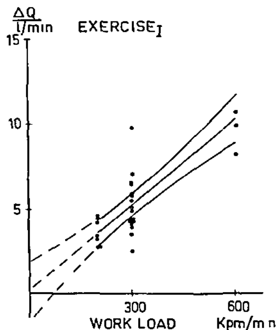


Fig 7 Diagram of cardiac output increase (ml) per kpm/min(ΔQ kpm/min) during the first (I) and second (II) exercise periods for 3 load groups



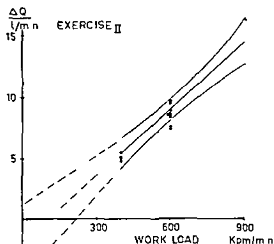
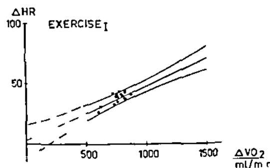


Fig. 8, 9. Linear regressions of cardiac output increase (y) on load (x) for the first (Fig. 8) and second (fig. 9) exercise periods. Eq. y = 0.0170x + 0.16 and y = 0.0182x — 1.90 respectively and r = 0.814 and 0.816. Symbols as in previous fig.



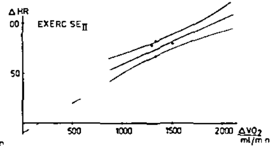
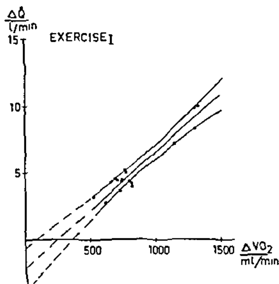


Fig. 10, 11. Linear regressions of heart rate increase (y) on oxygen consumption increase (x) during the first (fig. 10) and second (fig. 11) exercise periods. Eq. y = 0.0456x + 2.5 and y = 0.0439x + 14.4 respectively and r = 0.763 and 0.726. Symbols as in previous fig.



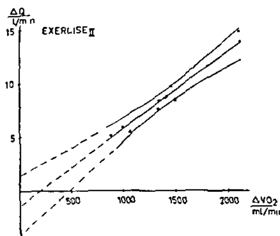


Fig 12 13 Linear regressions of cardiac output increase (y) on oxygen consumption increase (x) during the first (fig 12) and second (fig 13) exercise periods Eq $y = 0\,00896x - 2\,19$ and $y = 0\,00717x - 1\,40$ respectively and r 0 888 and 0 820 Symbols as in previous fig

the coefficients of correlation were calculated and tested No significant differences of the correlation coefficients for the various pairs of exercise periods were obtained for increases in oxygen consumptions, heart rate or cardiac output from rest The coefficients of the pooled groups were different The coefficient was higher for the oxygen consumption increases per load unit than for increases of heart rate and cardiac output per load unit ($P < 0\,03$ and $< 0\,05$ respectively) Within the different pairs of loads only that on 300 and 600 kpm/min had a higher coefficient of correlation for increase of oxygen consumption compared to increase of cardiac output ($P < 0\,04$)

The correlation between one variable and the increase of oxygen consumption was at least as good as the correlation between the same variable and work load in kpm/min, when increases of heart rate and cardiac output were considered (fig 10—13) The regression lines for increase of heart rate and increase of oxygen consumption on both loads had intersections with the y-axis not significantly deviating from the origin However, the line of cardiac output increase intersected significantly below the origin at the first load The fact that a relatively higher amount of the arteriovenous oxygen reserve is expended at lower loads might explain this The regressions of cardiac output increase on the increases of heart rate are shown in fig 14—15 The quotient calculations were justified The correlations between cardiac output and increase of heart rate and work load were not different

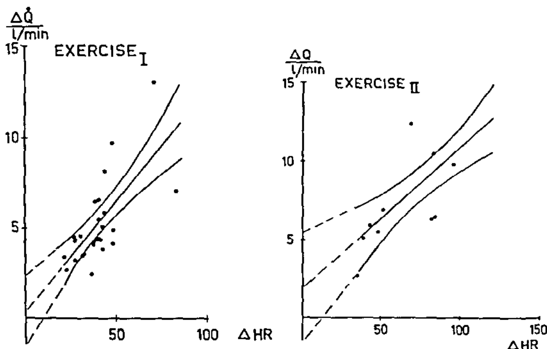


Fig 14 15 Linear regressions of cardiac output increase (y) on heart rate increase (x) on the first (fig 14) and second (fig 15) exercise period Eq $y = 0.124x + 0.36$ and $y = 0.0911x + 1.83$ respectively and r 0.735 and 0.630 Symbols as in previous fig

The second and third periods of exercise on the same load

In order to explore further the effect of previous exercise, the same load was repeated by 13 subjects seven at 600 kpm/min and six at 400 kpm/min See table IV and fig 16—18

Those exercising at 600 kpm/min had higher oxygen consumption increase during the repeated load (fig 16) When the two repeating load groups (400 kpm/min and 600 kpm/min) were pooled this was also evident

The heart rate increase during the repeated load was also higher for 600 kpm/min (fig 17) as well as when the two load groups were pooled The cardiac output increase however, was not changed when the load was repeated (fig 18)

The cardiac output increase per unit increase of oxygen consumption was lower during the repeated loads when the groups were pooled

The heart rate increase per unit oxygen consumption increase was not changed on the repeated load due to similar changes in the numerator and denominator

The cardiac output increase per unit increase of heart rate, (stroke volume change,) was smaller on the repeated load at 600 kpm/min as well as when the groups were pooled

Table IV *Increase of circulatory and respiratory variables during the second (II) and third (III) exercise periods on the same load For symbols see table II and III*

Loads (kpm/min)		n		Δ VO II	Δ VO III	Δ VO III II	Δ HR II	Δ HR III	Δ HR III II	Δ Q II	Δ Q III	Δ Q III II
II	III		$\bar{x}$	965	981	16	55 0	58 0	3 0	5 50	5 40	−0 10
400	400	6	s_x	62	36	43	20 1	19 7	4 0	1 55	1 47	0 50
			r	0 742			0 980			0 946		
			c v	6 4	3 7		36 7	34 0		28 4	27 2	
			$\bar{x}$	1434	1520	86	88 1	95 1	7 0	9 06	8 60	−0 46
600	600		s_x	75	73	54	15 2	20 6	5 7	2 01	1 57	1 18
		7	r	0 738		P < 0 01	0 994		P < 0 02	0 810		—
			c v	5 2	4 8		17,3	21 7		22 3	18 3	
				Δ VO / load			HR / load			Δ Q 10 3 / load		
			$\bar{x}$	2 41	2 50	0 09	0 143	0 152	0 010	14 45	13 95	0 50
Pooled			s_x	0 14	0 11	0 12	0 037	0 040	0 09	3 47	3 04	1 59
groups		13	r	0 983		P < 0 01	0 987		P < 0 005	0 935		—
			c v	5 8	4 4		25 9	26 3		24 0	21 8	

No differences between the variables per load unit at 400 and 600 kpm/min were obtained Thus only values for the pooled groups are given

The coefficients of variation of increases of oxygen consumption heart rate or cardiac output were not different when the loads were repeated

The correlation coefficient between the two loads at 400 kpm/min for cardiac output increase over increase of oxygen consumption was higher than for increase of oxygen consumption (P<0 02)

The group that repeated the load at 600 kpm/min also had different coefficients The best coefficient of correlation was that for increases of heart rate The probabilities that this coefficient was higher than those for increases of oxygen consumption, cardiac output, cardiac output over oxygen consumption and cardiac output over heart rate, were <0 01, <0 01, <0 01, and <0 04, respectively The coefficient of correlation for heart rate increase over oxygen consumption increase was higher than those for increase of oxygen consumption, cardiac output and cardiac output over oxygen consumption (P<0 02 <0 04, and<0 04, respectively)

In the pooled group, the coefficients of correlation were also different That for increases of oxygen consumption was

Table IV cont n

$\frac{\Delta Q \cdot 10^3}{\Delta VO_2}$ II	III	III II	$\frac{\Delta HR}{\Delta VO}$ II	III	III II	$\frac{\Delta Q}{\Delta HR}$ II	III	III II
5 72	5 54	−0 18	0 0576	0 0591	0 0016	0 10 9	0 0958	−0 00 1
1 62	1 63	0 50	0 0227	0 0195	0 0065	0 0341	0 0299	0 0151
0 993				0 964			0 896	
28 3	29 4		39 4	33 0		32 2	30 3	
6 29	5 66	−0 63	0 0617	0 0627	0 0010	0 1045	0 0976	−0 0119
1 30	0 99	0 76	0 0117	0 0138	0 0029	0 0273	0 0 04	0 0175
0 810		(P < 0 10)		0 989			0 902	P < 0 05
20 5	17 5		19 0	22 0		26 1	22 0	
6 03	5 61	−0 42	0 0598	0 0611	0 0013	0 1051	0 0954	−0 009
1 42	1 26	0 67	0 0170	0 0160	0 004	0 0293	0 0243	0 0134
0 882		P < 0 05		0 961			0 891	P < 0 025
23 5	22 5		28 3	26 2		27 8	25 4	

higher than those of increase of cardiac output over oxygen consumption and cardiac output over heart rate (P<0 03 and <0 04 respectively)

The difference between the coefficient of increases of heart rate and these variables were also significant (P<0 02 for both)

The coefficient of correlation between the variables on the second and third period at 400 l pm/min were compared with the corresponding coefficients between the first and second period at 200 and 400 kpm/min No difference were obtained for increases of oxygen consumption heart rate or cardiac output When similar comparisons were made between those who repeated 600 l pm/min and those who performed 300 and 600 kpm/min on the first and second loads a higher coefficient of heart rate increase was obtained when the loads were repeated (P<0 001)

The second and third periods of exercise with increasing load

Nine subjects in four different groups performed the second and third periods of exercise with increasing load The pooled results are given in table V and individual values in fig 19—24

Only one indicative difference between the coefficients of correlation for the variables was obtained the one for

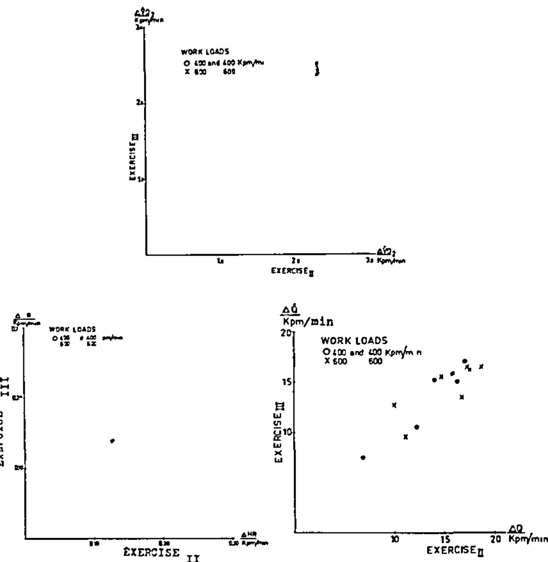


Fig 16 17 18 Diagrams of oxygen consumption increase (ml) per kpm/min (fig 16) heart rate increase per kpm/min (fig 17) and cardiac output increase (ml) per kpm/min (fig 18) during the second (II) and third (III) exercise periods at the same load Symbols as in previous fig

heart rate increase per load unit being higher than the one of cardiac output increase per load unit ($P<0\ 02$) Coefficients of correlation were not significant for increases per load unit of oxygen consumption and cardiac output Neither were there any significant correlations for cardiac output over oxygen consumption or cardiac output over heart rate The previously mentioned

Table V *I crea e of c rc latory a d respiratory tariables in n e s bjects d.ri g tle seco d (II) a d th rd (III) exercise period with i creas n, loa ls Loads 600 a d 900 kpm m n i i f e 900 a d 1200 kpm/m n in two 300 and 600 kpm m i i i o e and 200 a d 400 kpm/m n in one s bject For symbols see table II a d III*

	Δ VO load			Δ HR o d			Δ Q load			Δ Q 10³ / Δ VO₂			Δ HR / Δ VO₂			Δ Q / Δ HR		
	II	III	III II	II	III	III II	II	III	III II	II	III	III II	II	III	III II	II	III	III II
	2 3	2 33	−0 01	0 116	0 115	−0 001	14.39	12.81	1 58	6 70	5 49	0 1	0 0499	0 0494	−0 0005	0 1 83	0 1139	−0 0143
x	0 11	0 11	1 15	0 0 1	0 017	0 009	2 31	1 3	4	1 3	0 40	1 04	0 0091	0 00 8	0 0045	0 03 4	0 070	0 07 3
r	0 474			0 916			0 179	P	0 10	0 1	1 1	0 10	0 8 1			0 6_3		
	4 6	4 6		18 4	15 1		1 1	10 7		1 66	7.3		19 1	15		3	18 ?	

lower mechanical efficiency at lower loads and the different cardiac output response at lower load might explain this

*The increa es per load unit were com*pared with the corresponding variables in tho e who repeated the second load The oxygen consumption on the second load was not different but on the repeated load it was higher ($P<0\ 005$) The coefficients of variation were not different but the coefficient of correlation was higher when the load was repeated ($P<0\ 001$)

The increases of heart rate per load unit were higher in the group that repeated the load ($P<0\ 02$ and $P<0\ 005$) on the second and third load respectively The corresponding coefficients of variation or correlation were not different

The increase of cardiac output per load unit was not different when it was compared with the group that repeated the second exercise The coefficients of variation were also not different but the coefficient of correlation was higher when the loads were repeated ($P<0\ 005$) The increase of cardiac output per load unit and cardiac output over oxygen consumption had only indicative differences between the 2 exercise periods Comparison of cardiac output increase over oxygen consumption increase with the same variable in the group that re*peated the second exercise load revealed* no differences of mean values or coefficients of variation on the second period of exercise but on the third period the coefficient of variation was lower when the load was higher (7 3 and 22 5, respectively $P<0\ 05$) The mean values were not different, but the coefficient of correlation was higher when the second exercise load was repeated ($P<0\ 02$)

The increase of heart rate over oxygen consumption did not change with the higher loads (fig 23) Comparison with the second exercise load repeated revealed no differences of mean values or coefficients of correlation

Similar results were obtained for comparisons of increases of cardiac output over heart rate

Linear regression analyses of the changes of all the variables as dependent and load units as independent variables resulted in acceptable linearity and intersection with the y axis not significantly deviating from the origin for

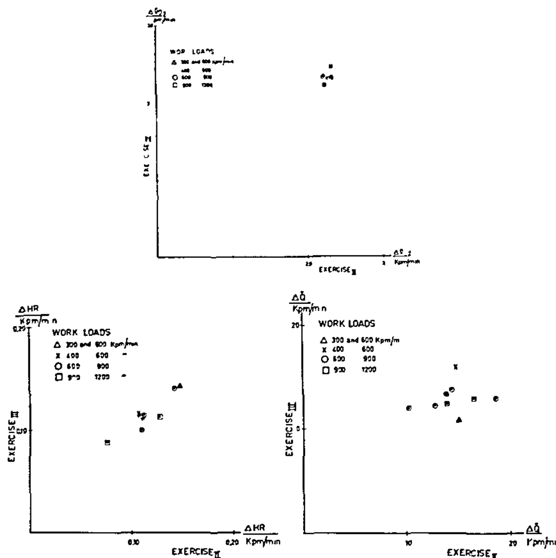


Fig 19 20 21 Diagrams of oxygen consumption increase (ml) per kpm/min (fig 19) heart rate increase per kpm min (fig 20) and cardiac output increase (ml) per kpm/min during the second (II) and third (III) exercise periods at increasing loads Symbols as in previous fig

both the second and third exercise periods

For the changes of the variables as dependent and increases of oxygen consumption and heart rate as independent variables linear regressions not deviating from the origin were found The correlation coefficients of the variables and increases of oxygen consumption or heart rate were at least as high as the corresponding coefficients of the variables and work load in kpm/min The quotient calculations were thus acceptable on both ways of calculation

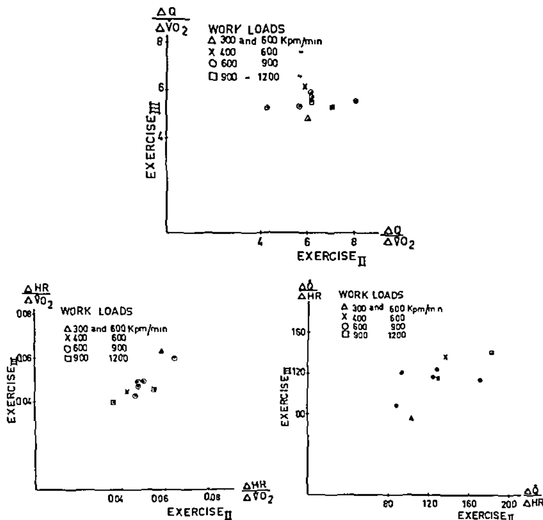


Fig 22 23 24 Diagrams of quotients of cardiac output increase (ml) over oxygen consumption increase (ml) (ΔQ/ΔVO) (fig 22) heart rate increase over oxygen consumption increase (ml) (ΔHR/ΔVO) (fig 23) and cardiac output increase (ml) over heart rate increase (ΔQ/ΔHR) (fig 24) during the second (II) and third (III) exercise periods at increasing loads

Discussion

The mean age of the group with the lowest load was significantly higher than the other two groups since the loads were chosen in accordance with the subjects physical fitness The groups did not differ with respect to height or weight The different age distributions in the groups could thus partly explain

c VI *Circulatory and respiratory variables during first (I) and second (II) exercise periods with increasing load For symbols see table*

oads (m min)	n		VO I	VO II	HR I	HR II	Q I	Q II	VO/load I	VO/load II	HR/load I	HR/load II	Q 10¹/load I	
II 400	7	$\bar{x}$	902	1228	96.3	116 0	9 87	11.26	4 509	3 070	0 482	0.290	4 94	2
		s_x	72	76	10 4	16 7	0 55	1 13	0 361	0 189	0 052	0 042	0 28	0
		r	0 901		0 937		0 787		P<0 001		P<0 001		P<0 0(	
		c v	8 0	6 2	10 8	14.3	5 5	10 1						
600	18	$\bar{x}$	1116	1750	106.5	148 4	11.31	15.31	3 720	2 917	0.355	0 247	3 77	2
		sx	170	242	12 1	16 1	2 22	2 47	0.563	0 403	0 040	0 027	0 74	0
		r	0 924		0 710		0 733		P<0 001		P<0 001		P<0 0(	
		c v	15.2	13 8	11 4	10 8	19 6	16 1						
900	4	$\bar{x}$	1652	2357	130 3	164 5	17 30	21 00	2 753	2 619	0 217	0 183	2 88	2
		sx	53	41	14 9	20 0	1 56	1 20	0 088	0 046	0 025	0 022	0 26	0
		r	0 718		0 941		0 962		P<0 025		P<0 005		P<0 0(	
		c v	3 2	1 7	11 4	12 2	9 0	5 7						
Pooled groups	29	$\bar{x}$							3 777	2 913	0 367	0 249	3 93	2
		sx							0 712	0.362	0 089	0 041	1 97	0
									P<0 001		P<0 001		P<0 0(	
		r							0 948		0 815		0 86(	
		c v							18 9	12 4	24.3	16 5	50 1	15

differences in the variables studied This might have been more important for the oxygen consumption due to the lower mechanical efficiency in older subjects (2) These age differences might have contributed to the displacement from the origin of the regression for increase of oxygen consumption due to the higher mean age among those who had lower loads

The effects of exercise on circulation and respiration are seen predominately in changes of blood flow, pressures heart rate ventilation and oxygen consumption The work performed is usually expressed in kpm or as a function of oxygen consumption to avoid the difficulties caused by different mechanical efficiencies at different loads Thus, the common method is to relate the variables studied to the work load or to a variable which is approximately linearly related to load such as oxygen consumption heart rate or cardiac output High correlations are found between these variables In the present study good correlations were also obtained when these methods were used (table VI)

To defend the suitability of using the increases from the rest values to express effects of exercise on the circulation and the respiration it is necessary that

ble VII *Circulatory and respiratory variables during the first (I) and second (II) exercise period with increasing load For symbols see table II and III SV stroke volume (l)*

Loads (kpm/min)	n		Q 10³ / VO I	Q 10³ / VO II	Q 10³ / VO II I	HR / VO₂ I	HR / VO₂ II	HR / VO₂ II I	Q / HR (SV) I	Q / HR (SV) II	Q / HR (SV) II I
II		$\bar{x}$	11 000	9 200	−1 800	0 1074	0 0950	−0 0124	0 1037	0 0987	−0 0050
) 400	7	s_x	1 020	1 068	0 723	0 0147	0 0177	0 0069	0 0131	0 0179	0 0057
		r	0 761		P<0 001	0 925		P<0 005	0 978		P<0 1
		c v	9 3	11 6		13 7	18 6		12 7	18 2	
		$\bar{x}$	10 144	8 806	−1.339	0 0965	0 0856	−0 0109	0 1068	0 1044	−0 0023
		s_x	1 295	1 261	1 258	0 0114	0 0111	0 0084	0 0194	0 0207	0 0154
) 600	18	r	0 515		P<0 001	0 722		P<0 001	0 705		
		c v	12 8	14.3		11 8	13 0		18 2	19 8	
		$\bar{x}$	10 475	8 925	−1 550	0 0788	0 0695	−0 0093	0 1335	0 1285	−0 0050
		s_x	0 814	0 386	0 533	0 0076	0 0078	0 0015	0 0086	0 0105	0 0101
0 900	4	r	0 840		P<0 02	0 981		P<0 005	0 457		
		c v	7 8	4 3		9 7	11 2		6 5	8.2	
		$\bar{x}$	10 397	8 917	−1 479	0 0967	0 0857	−0 0110	0 1097	0 1064	−0 0033
Pooled groups	29	s_x	1 202	1 120	1 069	0 0144	0 0144	0 0074	0 0192	0 0207	0 0128
		r	0 578		P<0 001	0 869		P<0 001	0 796		
		c v	11 8	12 6		14 9	14 9		17 5	19 4	

the correlations as well as the standard deviations are not different from those of the conventional methods

The hesitation to use change from rest values is probably due to the concept that rest values are much liable to disturbances due to the investigation procedure Anxiety of various degrees will disturb the variables more at rest than *during exercise (53) Reports using simi*lar quotients are however, available (17, 25, 39 40)

Between the two methods no differences of coefficients of correlation or standard deviations in the corresponding groups were obtained for oxygen consumption, heart rate or cardiac output The information on the effects of exercise was thus not significantly less when changes were used instead of the unabbreviated values

Similar calculations were made for the quotients, cardiac output over oxygen consumption heart rate over oxygen consumption and cardiac output over heart rate at increasing loads (table VII) *No differences of coefficients of* correlation were obtained The standard deviation of cardiac output over oxygen consumption for the second exercise period was smaller ($P<0\ 05$) than that of the corresponding quotient of increases For heart rate over oxygen consumption and cardiac output over heart

rate there were no differences of standard deviations The group mean values of cardiac output over oxygen consumption were not different on the first or the second exercise period They were different from the first to the second exercise period being smaller during the second period in each group When the increases with exercise were used, no such differences were obtained The mean values of the quotients heart rate over oxygen consumption were different for the first and second exercise periods Differences were also observed during the first exercise periods of the quotients cardiac output over heart rate The heart rates over oxygen consumption were smaller during the second period in each group, but for cardiac output over heart rate (stroke volume) no change was found When the calculations were based on the changes from rest values, the increase of cardiac output and heart rate over increase of oxygen consumption, and cardiac output increase over heart rate increase had no significant differences of standard deviations either for the *first or the second exercise period* Only one difference of mean values was obtained, i e oxygen consumption increase over heart rate increase for the first exercise The method using change from rest value was better than unsubtracted *values (when rest values are included)* to specify the effects of exercise when one load was followed by a second heavier load

The standard deviations of the unsubtracted values of oxygen consump tions per load unit were different in the 3 load groups on the first as well as on the second period of exercise, and the same results were obtained for cardiac output per unit load on the first period The heart rate per unit load on both periods and cardiac output per unit load on the second period had no significantly deviating standard deviations in the 3 load groups

The mean values of oxygen consumption per unit load and cardiac output per unit load were different on the first exercise period but not on the second The mean values of heart rate per unit load were different for both periods Of the variables expressing increase from the rest values only oxygen consump tion increase per unit load for the first exercise had different mean values in the 3 load groups

When the loads were repeated, the increases of oxygen consumption and heart rate were higher on the *repeated* load These changes also had linear regressions on age and body size (part IV) The difference of heart rate increase could thus partly be explained by lower mean age in the group that performed *600 kpm/min for two loads The blood* losses estimated at 50 ml per cardiac output determination were not substituted However, a drip with saline was used to flush the venous catheter and this replaced some of the loss The losses *might have influenced the heart rate* to some extent

When the loads of 600 kpm/min were repeated, the ventilatory exchange ratio was changed from an average of 0 98 to 0 94 ($P<0\ 001$), but the corresponding values at *400* kpm/min, *0 88* and *0 87*, were not different The pooled mean

values were different 0.93 and 0.91, respectively ($P<0.005$). When the ventilatory exchange ratio is smaller, more oxygen is needed per unit energy produced than when the ratio is higher. This might explain the higher oxygen consumption on the repeated load, when as seems probable, less carbohydrates in relation to fat are available. This is due to depletion of the glycogen stores by the preceding load (26). This decrease of ventilatory exchange ratio was not obtained when the next load was higher. Hypothetically this is due to less depletion by the lower load. The mean values for the pooled groups were 0.86 on the first and 0.91 on the second period of exercise ($P<0.001$). In each of the load groups differences were found. This might explain why less oxygen per unit load was consumed with the higher load. A higher work load may thus stimulate a higher utilization and mobilization of carbohydrates. Similar changes were obtained when the third exercise was at a higher level. The mean values were 0.86 and 0.92 ($P<0.001$) respectively, for the second and third period of exercise. In this case, however no change of oxygen consumption per unit load was obtained.

Since the cardiac output was unchanged when the second exercise load was repeated, the cardiac output increase over oxygen consumption increase and heart rate increase were lower on the repeated load.

When the third exercise load was at a higher level this difference was only indicative for cardiac output increase over heart rate increase. This variable was however, significantly lower when the higher load was in the second period of exercise.

These effects of a previous load on heart rate and oxygen consumption increase make it necessary to take into account the order of loads when responses to exercise repeated after half an hour of rest are evaluated. The degree of exercise is almost proportional to the increase of oxygen consumption, except at the smallest loads where the oxygen consumption increase per unit load is higher in mean value and standard deviation. Here, the mechanical efficiency and precision were lower than at higher load levels. In addition to the lower mechanical efficiency with lower loads, there were also tendencies to higher cardiac output increases and heart rate increases per unit load in comparison to the next load at a higher level.

The regression analyses showed that the quotient calculations were not justified for the increases of oxygen consumption and heart rate per unit load for the first bicycle exercise period (fig. 2—5). The quotient calculations with the increases of oxygen consumption or heart rate in the denominator were however, acceptable (fig. 10—15). Oxygen consumption increase and heart rate increase were better than loads in kpm/min as measures of work intensity when circulatory reactions at different loads were to be compared. Many practical problems such as calibrations of ergometers, different loads when the pedalling rate varies etc. are thus avoided.

Comparisons of the reactions on suc-

cessive increasing loads showed that the quotient heart rate increase over oxygen consumption increase was higher on the last load when the load was second in order The cardiac output increase over heart rate increase was lower when the higher load was second in order These quotients were unchanged when the higher load was third in order It thus seemed to be more differences between the first and second than between the second and third periods of exercise

In calculation great advantages are obtained when increases from rest values of the variables are used in comparison with handling both rest and exercise values simultaneously If they have linear relations not deviating from the origin with the oxygen consumption increase or heart rate increase these can be used in the denominator to relate changes of the other variable during exercise Four figures, the rest and exercise values for the numerator and the denominator are thus changed to one figure for each subject This variable can easily be used in regression analysis to explore the influence of various factors on exercise e g age, height and weight (part V) Some information may be lost as there was no indication of higher standard deviations for changes on higher loads (fig 10—15) This is outweighted by the comparable simplicity in calculations as compared to complicated covariance analysis which is another possibility

Summary

Changes from rest values of oxygen consumption, heart rate and cardiac out put during 10 minutes exercise periods on a bicycle ergometer were studied The purpose was to observe if changes per load unit were different on different loads of the same sequence of exercise and on different and same loads of different sequences of exercise Small changes were found

Linear regression analysis with the changes of the variables as dependent and loads as independent variables was made to see if quotient calculation of the changes over work load could be used Oxygen consumption and heart rate changes could not be properly used for quotient calculations

When the changes of a variable over changes of oxygen consumption or heart rate were tested similarly no objections to quotient calculations were obtained

Comparison of correlations between successive loads using change from rest values and the corresponding correlation of the unsubtracted values (when rest values were included) was made to see if precision was lost when change was used However, no precision was lost The standard deviations were also not changed

Quotient calculations with the unsubtracted exercise variables over work load, heart rate or oxygen consumption were not admissible

There were small but consistent diffe

rences in the changes from rest values during exercise on successive loads the changes being most pronounced between the first 2 exercise periods

The best results were obtained when the change of one variable per unit change of oxygen consumption was used

Great advantages in further regression analysis of the effect of exercise as independent and e g age and body size as independent variables are obtained when these quotients are used

General summary

Hemodynamic and respiratory variables were studied in 59 men, 17 to 59 years old and 28 women, 18 to 56 years old. Forty-three men and 21 women were paid volunteers and the remaining subjects were patients considered to have a normal circulation. Thirty-six men and nineteen women had mainly labor work and the others sedentary jobs. Regular physical training was reported by 17 men and four women.

The studied variables were heart rate, intraarterial blood pressures, cardiac output, hematocrit and from expired air pulmonary ventilation, oxygen consumption and ventilatory exchange ratio. From these variables systemic vascular resistance and conductance, left ventricular work and stroke work, arteriovenous oxygen difference, were also calculated. These variables were recorded at rest sitting in an arm chair and thereafter during exercise sitting on a bicycle.

In 24 men and 20 women recordings at rest were also made in the recumbent position before the determinations in the sitting position. The recordings in the sitting position were repeated by 14 men before any exercise. The work load on the bicycle was repeated by 13 men. Successive tests on increasing loads were also made in some subjects.

The aim of the study was to obtain information on how these variables change with age and body build in normal men and women. Linear regression analyses were used systematically. The variables were tested to see if differences in body build or age could explain the sex differences obtained.

Rest in recumbent position

Weight had positive correlations with pulse pressure in men and with diastolic pressure in women. Body surface area had a positive correlation with diastolic pressure in women. Age had positive correlations with systolic and mean pressure in women.

Cardiac output in men had positive correlations with height and body surface area and a correlation with the combination of age and body surface area. Stroke volume, left ventricular work and stroke work in men had positive correlations with weight and body surface area. In women only left ventricular work had similar correlations with weight and body surface area. Systemic vascular resistance of men had negative correlations with height and body surface area. The combination of age and body surface area had correlations with resistance in men and women. For the systemic vascular conductance these correlations became positive.

The oxygen consumption of men and women had positive correlations with weight and body surface area. In women

a correlation with height was also obtained The pulmonary ventilation of men was positively correlated with height

The correlations were not strong The correlation coefficients ranged in men from 0 691 for left ventricular stroke work and weight to 0 414 for left ventricular work and weight and in women from 0 577 for oxygen consumption and height to 0 452 for diastolic pressure and body surface area

In men mean values were higher for cardiac output stroke volume left venticular work and stroke work, conductance, oxygen consumption, pulmonary ventilation and hematocrit and lower for pulse pressure and resistance Stroke volume, left ventricular stroke work, oxygen consumption and pulmonary ventilation had higher variances in men

After adjustments with the regressions on age and/or body size the sex differences for cardiac output, stroke volu me, left ventricular work and stroke work, systemic vascular resistance and conductance and pulmonary ventilation disappeared The sex differences for pulse pressure and oxygen consumption remained after these adjustments

Rest in the sitting position

Age had positive correlations with systolic, mean and pulse pressure in women In men positive correlation was obtained only for mean pressure Body size had no influence on pressures

In men age had negative correlations with cardiac output stroke volume systemic vascular conductance and positive correlation with systemic vascular resistance In women age had positive correlation with resistance and negative correlation with conductance Height of men had positive correlations with cardiac output, stroke volume and conductance and a negative correlation with resistance Weight of men had positive correlations with stroke volume and left ventricular stroke work Body surface area of men had positive correlations with cardiac output, stroke volume, left ventricular stroke work conductance and a negative correlation with resistance Combinations of age *and body size in men had correlations* with cardiac output, stroke volume systemic vascular resistance and conductance Body size in women had no influence on these variables

Age had no effect on ventilation and oxygen consumption Age of women had positive correlations with arteriovenous oxygen difference and hematocrit Height, weight and body surface area had positive correlations with oxygen consumption in men and women and positive correlations with pulmonary ventilation in men Weight and body surface area of women were positively correlated with arteriovenous oxygen difference

The correlation coefficients were rather small In men they ranged from 0 593 for age combined with height and resistance to 0 282 for age and mean pressure In women they ranged from 0 546 for body surface area and oxygen consumption to 0 374 for age and hematocrit

In men mean values were higher for cardiac output stroke volume, left ven-

tricular work and stroke work, conductance, oxygen consumption, pulmonary ventilation and hematocrit and lower for resistance In men the variance of left ventricular work was higher and the variance of hematocrit was lower than in women Adjustments according to the regressions on age and/or body size, resulted in disappearence of sex differences for cardiac output, stroke volume, left ventricular stroke work, systemic vascular resistance and conductance and pulmonary ventilation The differences of oxygen consumption and hematocrit remained

From recumbent to sitting position

In women the responses to sitting for heart rate and systemic vascular resistance had correlations with age The heart rate response of women also had a correlation with height In men the response of pulmonary ventilation had correlations with weight and body surface area High correlations between the corresponding recumbent and sitting variables were obtained in men and women The correlation coefficients were highest for hematocrit (0 961 and 0 978 respectively) The lowest correlations coefficient were 0 598 for ventilatory exchange ratio in men and 0 505 for arteriovenous oxygen difference in women

Sex difference of the mean values for responses to sitting was obtained only for hematocrit which increased more in men The variances of responses in men were higher for systolic and mean pressures stroke volume left ventricular stroke work and pulmonary ventilation In women the systolic and mean pressure decreased when sitting In men pulse pressure decreased when sitting In men and women the cardiac output, stroke volume, left ventricular work and stroke work and systemic vascular conductance decreased The resistance and arteriovenous oxygen difference increased when sitting

Reproducibility at rest and during exercise

The differences between the repeated variables had correlations with age and body size Age had correlations with the differences between repeated determinations of oxygen consumption and pulmonary ventilation at rest and of heart rate during exercise Weight and body surface are had correlations with the corresponding difference of oxygen consumption during exercise The heart rate difference at rest had a correlation with body surface area

The mean value of the repeated measurement for heart rate at rest was lower During exercise the repeated measurements were higher for heart rate, oxygen consumption and arteriovenous oxygen difference and lower for stroke volume, left ventricular stroke work and ventilatory exchange ratio

The variances of the repeated measurements were not different from those at the first measurements

High correlations between the repeated variables were common At rest, the coefficients ranged from 0 962 for diastolic pressure to 0 059 for ventilatory exchange ratio The next lowest coefficient was 0 429 for oxygen consumption Only these two correlation coefficients

were insignificant During exercise significant coefficients were obtained for all the variables The range was from 0 985 for heart rate to 0 804 for arteriovenous oxygen difference

Exercise sitting on a bicycle

The exercise variable used was the rest value in the sitting position subtracted from the exercise value over the corresponding increases of oxygen consumption and heart rate

Of the variables over oxygen consumption increase, heart rate response in women had negative correlations with height and body surface area The systolic and pulse pressure responses in men had positive correlations with age Stroke volume response in women had positive correlation with height Systemic vascular resistance response in men had negative correlation with age and positive correlations with height and body surface area The resistance response of women also had negative correlation with age Age combined with body size in men had correlations with responses of resistance and arteriovenous oxygen difference

The correlation coefficients of men varied between 0 508 for age combined with body surface area and arteriovenous oxygen difference response and (—)0 264 for age and systemic vascular resistance response The range in women was from (—)0 614 for heart rate response and body surface area to (—) 0 424 for resistance response and age

Women had higher mean responses than men for heart rate, systolic mean and pulse pressure cardiac output systemic vascular conductance, arteriovenous oxygen difference and hematocrit and a lower mean response of resistance The variances of response were lower in men for heart rate, arterial pressure cardiac output resistance and conductance, ventilatory exchange ratio and arteriovenous oxygen difference The adjustments according to the regressions on age and/or body size resulted in disapperance of sex differences for responses of heart rate, systolic pressure and arteriovenous oxygen difference but the differences remained after adjustments of responses for pulse pressure resistance and conductance

Of the variables per unit heart rate increase, the responses of men for systolic, mean and pulse pressure had positive correlations with age The cardiac output response of women had positive correlations with age, height and body surface area Stroke volume responses of men and women had positive correlations with age and women also had positive correlations with height weight and body surface area The systemic vascular resistance response for men and women had negative correlations with age In men it had a positive correlation with height and in women it had negative correlations with weight and body surface area Age combined with body size and resistance response had correlations in men The response of conductance in women had positive correlations with age, height and body surface area The response of oxygen consumption in women had positive correlations with age weight and body surface area The responses of pulmonary ventilation and arteriovenous oxygen difference in wo-

men had positive correlations with weight and body surface area The response of arteriovenous oxygen difference in men had a positive correlation with age

The response of hematocrit and body surface area in women was positively correlated

The mean values of the response per unit increase of heart rate were higher in men for systolic pressure, cardiac output, oxygen consumption and pulmonary ventilation The variance of the responses of resistance was lower in men Adjustments for differences in age or body size resulted in disappearence of sex differences in response of cardiac output and oxygen consumption New sex differences were revealed after adjustment according to the regressions for responses of pulse pressure, stroke volume systemic vascular resistance and conductance, arteriovenous oxygen difference and hematocrit The sex difference of response for cardiac output was even reversed after adjustment according to the regression on height

The variation in response was generally lower for the variable per unit oxygen consumption increase than the corresponding variable per unit heart rate increase In men the coefficients of variation for response per unit oxygen consumption increase were numerically lower in 10 out of 12 variables and in women in 9 out of 12 variables The responses of ventilatory exchange ratio and hematocrit in men and systolic pressure stroke volume and conductance in women had lower coefficients of variation per unit heart rate increase than per unit oxygen consumption increase

Responses to different work loads

A first load (200—600 kpm/min) and a second higher load (400—900 kpm/min) were performed by 29 men The responses of oxygen consumption, heart rate and cardiac output were analysed On successive loads at the higher levels the oxygen consumption increase paralleled the load increase but on low levels the oxygen consumption increase was higher per unit work load at the first lower load Thus on the first load, those with the lowest load level had the highest oxygen consumption increase per load unit There were linear relationships between load and oxygen consumption increase for both periods of exercise The heart rate increase per load unit was highest for the lowest load (200 kpm/min) The other loads had similar heart rate increase per load unit The first load had a linear relation between load and heart rate increase but not the second The cardiac output increase per load unit was also highest on the smaller load compared with the second higher load Linearity between load and cardiac output increase, was, however, acceptable on both loads The correlation between one variable and oxygen consumption increase was at least as high as the correlation between the same variable and work load Quotients using the increase of one variable over the corresponding increase of oxygen consumption or heart rate were admissible except for cardiac output increase over oxygen consumption increase on the first load

The second and third periods of exer-

cise were made on the same load by 13 men Those repeating the higher load (600 kpm/min) had higher increase of oxygen consumption and heart rate in the repeated period of exercise The cardiac output increases per unit increase of oxygen consumption and heart rate were therefore lower during the repeated exercise

The second and third periods of exercise with increasing load were made by nine men A higher variability of response was obtained in this group Linearity between work load and increase of the variables was obtained Oxygen consumption increase and heart rate increase also had linear relations with the other variables The correlation between one variable and increase of oxygen consumption was at least as good as the correlation between the same variable and work load Quotient calculations of increases of one variable over work load, increases in oxygen consumption and heart rate were justified

The variances of the variables expressing increase were not different from those of the corresponding unsubtracted variables

It was not admissible to use the unsubtracted variable per unit work load, oxygen consumption or heart rate, due to deviations from origin of the intersections of the corresponding regression lines

Acknowledgements

The subjects in the study have willingly cooperated during the complicated procedure lasting from 8 o clock a m to about noon It included discomfort from fast, catheters and efforts during exercise The inpatients were not paid and the other subjects received a small reward as a compensation for loss of one day s work My thanks go to them

Considerable help was provided by my collegues at the First Medical Service, Sahlgren s hospital in the performance of the investigations and skilled assistance was given by the laboratory and secretarial staff My chiefs Professor Lars Werko and Assistant Professor Ed Varnauskas have supported the work and lent valuable criticism

My teachers in statistics from the University of Gothenburg, especially fil lic Esbjorn Carlstrom, have taken much interest in the study and have aided in the choice of statistical methods and computer work

M D Russel Luepker and M D David Lewis have commented the papers and corrected my english text

The laboratory work has been supported with money from the Swedish National Association against Heart and Chest Diseases The fees for computer time were paid by Statskontoret My sincere thanks are given to all institutions and persons who helped in this study

References

1 Asmussen E & Mathiasen P Some physiologic functions in physical education students re investigated after twentyfive years J Amer Geriat Soc *10* 379 1962

2 Åstrand I Aerobic work capacity in men and women with special reference to age Acta physiol scand *49* Suppl 169 1960

3 Åstrand P O Experimental studies of physical working capacity in relation to sex and age Munksgaard Copenhagen 1952

4 Åstrand P O Cuddy T E Saltin B & Stenberg J Cardiac output during submaximal and maximal work J appl Physiol *19* 268 1964

5 Becklake M R Frank H Dagenais G R Ostiguy G L & Guzman C A Influence of age and sex on exercise cardiac output J appl Physiol *20* 938 1965

6 Bevegård S Holmgren A & Jonsson B The effect of body position on the circulation at rest and during exercise with special reference to the influence on the stroke volume Acta physiol scand *49* 279 1960

7 Bjure, J Pulmonary diffusing capacity for carbon monoxide in relation to cardiac output in man Scand J clin Lab Invest *17* Suppl *81* 1965

8 Boothby W M Berkson J & Dunn H L Studies of energy of metabolism of normal individuals standard for basal metabolism, with nomogram for clinical application Amer J Physiol *116* 468 1936

9 Brandfonbrener M Landowne M & Shock N W Changes in cardiac output with age Circulation *12* 557 1955

10 Brownlee, K A Statistical theory and methodology in science and engineering 2nd ed Wiley New York London and Sydney *1965*

11 Chapman C B Fisher J N & Sproule B J Behavior of stroke volume at rest and during exercise in human beings J clin Invest *39* 1208 1960

12 Dahlberg G Statistical methods for medical and biological students Georg Allen & Unwin London 1948

13 Das B C & Mukherjee B N Variation in systolic and diastolic pressure with changes in age and weight Gerontologia (Basel) *8* 92, 1963

14 Davies O L The design and analysis of industrial experiments Oliver & Boyd London 1954

15 Du Bois D & Du Bois E F A formula to estimate the approximate surface area if height and weight be known Arch intern Med *17* 863 1916

16 Forsberg S Å Pulmonary blood volume in man A study using the double indicator technique in patients with cardiovascular disease Acta med scand *175* Suppl 410 1964

17 Foster G L Reeves T J & Meade, J H Jr Hemodynamic responses to exercise in clinically normal middle aged men and in those with angina pectoris J clin Invest *43* 1758 1964

18 Granath A Jonsson B & Strandell, T Circulation in healthy old men studied by right heart catheterization at rest and during exercise in supine and sitting position Acta med scand *176* 425 1964

19 Granath A & Strandell, T Relationships between cardiac output, stroke volume and intracardiac pressures at rest and during exercise in supine position and some anthropometric data in healthy old men Acta med scand *176* 447 1964

20 Grimby G Nilsson N J & Saltin B Cardiac output during submaximal and maximal exercise in active middle aged athletes J appl Physiol *21* 1150 1966

21 Hamilton W F & Remington J W Some factors in the regulation of the stroke volume Amer J Physiol *153* 287 1948

22 Hamilton W F Moore, J W Kinsman J M & Spurling R G Studies on the circulation IV *Further analysis of the injection* method and of changes in hemodynamics under physiological and pathological conditions Amer J Physiol *99* 534 1932

23 Hanson J S Tabakin B S & Levy A M Comparative exercise-cardiorespiratory performance of normal men in the third fourth and fifth decades of life Circulation *37* 345 1968

24 Harris E A & Thomson J G The pulmonary ventilation and heart rate during exercise in healthy old age Clin Sci *17* 349 1958

25 Harvey R M Ferrer M I Richards D W & Cournand A Cardiocirculatory performance in atrial flutter Circulation *12* 507 1955

26 Hermansen L Hultman E & Saltin B Muscle glucogen during prolonged severe exercise Acta physiol scand *71* 129 1967

27 Holmgren A Circulatory changes during muscular work in man With special reference to arterial and central venous pressures in the system cic circulation Scand J clin Lab Invest *8* Suppl 24 1956

28 Holmgren A Jonsson B & Sjöstrand T Circulatory data in normal subjects at rest and during exercise in recumbent position with special reference to the stroke volume at different work intensities Acta physiol scand *49* 343 1960

29 Holmgren A & Mattsson K H A new ergometer with constant work load at varying pedalling rate Scand J clin Lab Invest *6* 137 1954

30 Jegier W Sekelj P Auld P A M Simpson R & McGregor M The relation between cardiac output and body size Brit Heart J *25* 425 1963

31 Julius S Amery A Whitlock L S & Conway J Influence of age on the hemodynamic response to exercise Circulation *36* 222 1967

32 Knuttgen H G Aerobic capacity of adolescents J appl Physiol *22* 655 1967

33 Lagerlof H Eliasch H Werko L & Berglund E Orthostatic changes of the pulmonary and peripheral circulation in man Scand J clin Lab Invest *3* 85 1951

34 Lee T D Jr Lindeman R D Yiengst M J & Shock N W Influence of age on the cardiovascular and renal response to tilting J appl Physiol *21* 55 1966

35 Lund-Johansen P Hemodynamics in early essential hypertension Acta med scand Suppl 482 1967

36 Mellette, H C Boot R W Ryan J M & Rieser G F The use of bromsulphalein (B S P) as an indicator for the determination of cardiac output by the dye dilution technique J Lab clin Med *51* 441 1958

37 Moore P G Regression as an analytic tool Applied Statistics *9* 106 1962

38 Naimark A Wasserman K & McIlroy M B Continuous measurement of ventilatory exchange ratio during exercise J appl Physiol *19* 644 1964

39 Nowy H Kikodse, K & Zöllner N Uber Bestimmungen des Herzminutenvolumens und zentralen Blutvolumens in Ruhe und bei korperlicher Arbeit mit Hilfe der Farbstoffmethode Z Kreisl Forsch *46* 382 1957

40 Podlesch I & Ulmer W T Über die Abhangigkeit von Herzminutenvolumen Herzindex Schlagvolumen Schlagvolumenindex und Sauerstoffverbrauch vom Lebensalter Arch Kreisl Forsch *48* 232 1965

41 Reeves J T Grover R F Blount S G Jr & Filley G F Cardiac output response to standing and treadmill walking J appl Physiol *16* 283 1961

42 Scholander P F Analyzer for accurate estimation of respiratory gases in one half cubic centimeter samples J biol Chem *167* 235 1947

43 Seldinger S I Catheter replacement of the needle in percutaneous arteriography A new technique Acta radiol (Stockholm) *39* 368 1953

44 Smith W W Wikler N S & Fox A C Hemodynamic studies of patients with myocardial infarction Circulation *9* 352 1954

45 Sulyan H Vincent W A Kasheisianp U Cuddy R P & Eich R H An evaluation of the cardiac index Amer Heart J *72* 621 1966

46 Snedecor G W Statistical methods 5th Ed Iowa State University Press Ames Iowa 1959

47 Stewart G N Researches on the circulation time and on the influences which affect it IV The output of the heart J Physiol (London) *22* 159 1897—98

48 Strandell, T Circulatory studies on healthy old men Acta med scand *175* Suppl 414 1964

49 Tabakin B S Hanson J S Merriam T W Jr & Caldwell E J Hemodynamic response of normal men to graded treadmill exercise J appl Physiol *19* 457 1964

50 Thomasson B A study in right heart performance in man with special reference to the effect of experimentally induced acute hypervolemia Scand J clin Lab Invest *11* Suppl 40 1959

51 Truedsson E Variation of arterial blood pressure with age sex anthroposomatological dimensions plasma lipids in the fasting state and after fat ingestion Acta med scand *172* Suppl 381 1962

52 Tuckman J & Shillingford J Effect of different degrees of tilt on cardiac output heart rate and blood pressure in normal man Brit Heart J *28* 32 1966

53 Wade O L & Bishop J M Cardiac output and regional blood flow Blackwell Scientific Publications Oxford 1962

54 Wang Y Marshall, R J & Shepherd J T The effect of changes in posture and of graded exercise on stroke volume in man J clin Invest *39* 1051 1960

55 WARD R J DANZIGER F BONICA J J ALLEN G D & TOLAS A G Cardiovascular effects of change of posture Aerospace Med *37* 257 1966

56 WASSEN A The use of bromsulphalein for determination of the cardiac output Scand J clin Lab Invest *8* 189 1956

57 WASSERMAN K VAN KESSEL A L & BURTON G G *Interaction of physiological mechanisms* during exercise J appl Physiol *22* 71 1967

58 WELCH B L Further note on Mrs Aspin s tables and on certain approximations to the tabled function Biometrika *34* 293 1949

59 WIDIMSKY J BERGLUND E & MALMBERG R Effect of repeated exercise on the lesser circulation J appl Physiol *18* 983 1963

60 YATES F Sampling methods for censuses and surveys 2nd Ed London 1953

Errata

(p = page, rc = right column, lc = left column, ln = line (headlines not included)
The corrections come after the present expressions)
p 6, lc, ln 9 influences — influences, rc, ln 3, have — has
p 14, rc, ln 8, ventricular — ventricular stroke
p 21, rc, ln 30, are — is
p 22 lc ln 6, had — has
p 27, ln 3, computor — computer
p 49, rc, ln 12, was — were
p 51—53 $\sum \frac{(x_2 - x_1)^2}{2n}$ — $\sqrt{\frac{\Sigma(x_1 - x_2)^2}{2n}}$
p 67, ln 1 confidencelimits — confidence limits
p 73 fig 12, 0 —15, —10 (scale wrong) — 0, —5, —10
p 76, lc, ln 26, $(a-\bar{v})_{O_2}$ — $(a-\bar{v})_{O_2}$
p 87, lc ln 8, change — changes, ln 9, change — changes
p 98, table IV, $\frac{IQ \; 10^3}{load}$ — $\frac{AQ \; 10^3}{load}$

ta Medica Scandinavica

ementum 496

sine Poisoning
Metal Refining Plant

een Simultaneous Cases

[illegible]

[illegible] Studies

cal Picture and Treatment in Arsine Poisoning

termination of Arsenic in Biological Material by Radioactivation Analysis

e Renal Circulation in Arsine Poisoning

by Bent Nielsen

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv*, was founded in 1869 by Professor Axel Key, MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German Acta Medica Scandinavica is published on a non-profit basis

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each a year) include free supplements to the current volumes During the past few years an average number of 17 supplements has been issued per annum

Subscribtion Rates

Per annum = two volumes
In Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Sw kr 120 incl. postage
Other countries Sw kr 140, incl postage

Chief Editor

Associate Professor Birger Strandell, MD
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Biblioteksgatan 11
S-111 46 Stockholm Sweden

Subscription and Distribution

Almqvist & Wiksell
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1, Sweden

Arsine Poisoning
in Metal Refining Plant

Fourteen Simultaneous Cases

Acta Medica Scandinavica

Supplementum 496

Arsine Poisoning in Metal Refining Plant

Fourteen Simultaneous Cases

Edited by Bent Nielsen

Distributed by Almqvist & Wiksell Stockholm, Sweden

The arsine poisoning acc
present papers occurred in
SON's metal refining pla
in the month of March 1965

A donation from SVEND BERGSØE's FUND made it possible to publish the papers as a supplementum to Acta Medica Scandinavica

The authors wish to express their thanks to the fund

Arsine Poisoning in Metal Refining Plant

Fourteen Simultaneous Cases

I *Introduction*

By

Poul Anthonisen Bent Baunøe † Børge Fallentin Jørgen Frost Aage Grut Lis Vinther Kristensen Jørgen Ladefoged Ole Munck Bent Nielsen Fritz Pedersen Kirsten Pedersen Flemming Raaschou Jørgen Thomas & Kjeld Winkler

II *Environmental Studies*

By

Børge Fallentin Jørgen Frost & Aage Grut

III *Clinical Picture and Treatment in Arsine Poisoning*

By

Poul Anthonisen Bent Nielsen Kirsten Pedersen & Flemming Raaschou

IV *Determination of Arsenic in Biological Material by Radioactivation Analysis*

By

Jørgen Thomas & Lis Vinther Kristensen

V *The Renal Circulation in Arsine Poisoning*

By

Fritz Pedersen Jørgen Ladefoged Kjeld Winkler Bent Baunøe † & Ole Munck

ARSINE POISONING IN METAL REFINING PLANT

Fourteen simultaneous cases

I

INTRODUCTION

by

Poul Anthonisen B Baundøe † Børge Fallentin Jørgen Frost Aage Grut
Lis Vinther Kristensen J Ladefoged O Munck Bent Nielsen F Pedersen
Kirsten Pedersen Flemming Raaschou Jørgen Thomas & K Winkler

Arsine poisoning is a typical example of industrial intoxication and arsine appears to be that arsenic compound which has given rise to the most serious cases of poisoning during industrial processes

Muehlberger et al (8) noted a total of 247 reported cases during the period 1815 to 1928 50 of which terminated fatally In a monograph from 1962 *Buchanan* (1) has reviewed the circumstances under which arsine gave rise to poisoning Arsine can be produced by the reduction of arsenic compounds with hydrogen or by hydrolysis of arsenides An example of the former process which might be mentioned is a case during the First World War where in two British submarines with a total crew of 56 respectively 27 and 28 men were poi oned as a result of a reduction of the arsenic in the lead plates of the accumulators (3) Hydrolysis of arsenides is considered to be the cause of the formation of arsine in the refining of tin and similar processes and it is these working processes in particular which in recent years have given rise to arsine poisoning (1) Thus a report prepared by the European Tin and Lead Smelters Club in 1965 (10) states that in the associated enterprises during the years 1922–1965 there were at least 87 cases of arsine poisoning Twenty four of these patients died

In Scandinavia *Dalgaard & Gregersen* (2) have described arsine and phosphine poisoning during the shipment of ferro-silicon which had become wet

The clinical picture in arsine poisoning is characterized by haemolysis in contrast to the poisoning with other arsenic compounds

Various theories have been proposed to explain the haemolysis *Flury & Zernik* (4) considered that arsine is first bound to the haemoglobin in the erythrocytes and that haemolysis then develops *Hunter et al* (6) subsequently showed that 95–99 % of the arsenic is found bound to the erythrocytes *Jung* (7) on the basis of his own studies and a review of the literature has proposed four factors which may explain the haemolysis 1) the haemolytic effect of elementary arsenic 2) inhibition of catalase by arsenic or arsenic compounds 3) the haemolytic effect of hydrogen peroxide formed by the oxidation of arsine and 4) the destruction of the haemolysis-inhibiting effect of glutathione The last theory is supported by *Pernis & Magistretti* (9) *Goodman & Gilman* (5) concluded in 1965 that the mode of action of arsine is not fully elucidated

On the 24th 25th and 26th of March 1965 5 patients were admitted to the Copenhagen Kommunehospital and 9 patients to Glostrup Hospital with such a uniform disease picture that it was soon suspected that they had all been exposed to the same agency—a suspicion which was strengthened by the fact that all the patients worked in the same industrial enterprise As will appear from the following these cases were in all probability examples of poisoning with arsine (AsH_3)

In the following papers, a description will be

given of the problems of industrial hygiene (II) the course of the poisoning in the 14 patients (III) the radioactivation technique used to determine arsenic in various tissues (IV) and the results of renal physiological studies performed in 2 of the patients (V)

REFERENCES

1 Buchanan W D Toxicity of Arsenic Compounds p 88 Elsevier Amsterdam London New York 1962

2 Dalgaard, J B & Gregersen M Forgiftningsfaren under sotransport af ferrosilicium Nord Med 71 271 1964

3 Dudley S F Toxemic Anemia from Arseniuretted Hydrogen Gas in Submarines J Indust Hyg 1 215 1919

4 Flury F & Zernik, F Schädliche Gase Springer Berlin 1931

5 Goodman L. S & Gilman A The Pharmacological Basis of Therapeutics, p 948 III Ed the Macmillan Company New York London & Toronto 1965

6 Hunter F T., Kip A F & Irvine, J W Radioactive Tracer Studies on Arsenic Injected as Potassium Arsenite J Pharmacol Exp Therap 76 207 1942

7 Jung F Loslichkeit und Reaktionsweise des Arsenwasserstoffs im Blut Biochem Ztschft., 30? 294 1939

8 Muehlberger C W., Loevenhart A S & O'Malley T S Arsine Intoxication A Case of Suspected Poisoning in the Steel Industry J Indust Hyg 10 137 1928

9 Pernis, B & Magistretti M A Study of the Mechanism of Acute Hemolytic Anemia from Arsine Med Lavoro 51 37 1960

10 Rey M European Tin and Lead Smelters Club Report on "Arsine Poisoning in the Refining of Tin Alloys" Ecole Nationale Supérieure des Mines de Paris, France 1965

II

ENVIRONMENTAL STUDIES

by

Børge Fallentin Jørgen Frost & Aage Grut

From the State Institute of Occupational Hygiene Copenhagen (Chief B Fallentin)

The Clinic for Occupational Diseases Rigshospitalet Copenhagen (Chief J Frost M D)

Factory Inspectorate Medical Services (Chief Aage Grut M D)

On Thursday the 25th March 1965 at about 3 30 p m the Factory Inspectorate Medical Services received a report from two hospitals (Copenhagen Kommunehospital and Glostrup Hospital) to the effect that on the same day several workmen from a metal refining plant had been admitted with symptoms of haemolysis The Factory Inspectorate was informed and likewise the District Medical Officer of Health as according to the first information available the patients had been working at widely different sites in the plant so that the possibility of non industrial poisoning existed As the examination of the canteen by the District Medical Officer did not point to food poisoning attention was directed that same evening to the fact that arsine inhalation was the most likely cause and at 9 p m it was decided to stop production During the course of the evening those workmen who had reported sick on the day in question were encouraged to see their doctor to be admitted to hospital if necessary likewise the radio news bulletin was used to request those workers at the factory who had nausea headache or dark urine to do the same Numerous meetings were held between the staff of the plant and the Factory Inspectorate to investigate the possible causes of poisoning Production details for the 25th of March and the days preceding were reviewed and those poisoned were questioned as to their work and movements

Cause

Based on the clinical symptoms arsine or possibly stibine or a combination of these had to be regarded as the most likely cause of the poisoning A determination of arsenic*) was made by the State Institute of Occupational Hygiene in 39 portions of urine from 11 of those poisoned During the first few days excretion was between 0 6 and 5 8 mg As_2O_3/l on discharge it was between 0 2 and 0 4 mg/l and 6 months later it varied from 0 to 0 1 mg/l Figure 1 shows the excretion in two patients who were followed during the course of hospitalization As it must be assumed that the workmen may be exposed to the breathing of dust containing arsenic under normal working conditions and since cases have been described of excretion of several mg arsenic per 24 hours without the subject in question developing any symptoms of poisoning the excretion of arsenic in urine is not in itself a definite proof of poisoning

A fortnight after the accident samples of urine from seven workmen with working places adjacent to those of the poisoned were analysed and showed an excretion of less than 0 1 mg As_2O_3/l

This suggests that those poisoned has suffered an exceptional exposure to arsenic and supports the assumption of arsine poisoning Studies of other biological material from the patients also showed an increased content of arsenic while antimony was not demonstrated (14)

On the 26th of March, the factory was inspected Air analysis by Drager Multi Gas Detector showed no arsine at any location

*) After the destruction of the urine by means of nitric acid and sulphuric acid the arsenic content is determined by Gutzeit s method (2 6)

given of the problems of industrial hygiene (II) the course of the poisoning in the 14 patients (III) the radioactivation technique used to determine arsenic in various tissues (IV) and the results of renal physiological studies performed in 2 of the patients (V)

REFERENCES

1 Buchanan W D Toxicity of Arsenic Compound p 8? Elsevier Amsterdam London New York 196

2 Dalgaard J B & Gregersen M Forgi n r sfaren under sstran port af ferrosili ium Nord Med *71* 271 1964

3 Dudley S F Toxemi An mia from Arseniuretted Hydrogen Gas in Submarines J Indust Hyg *1* 215 1919

4 Flury F & Zernik, F Schädliche Gase Springer Berlin 1931

5 Goodman L S & Gilman A The Pharmacological Basis of Therapeutics, p 948 III Ed., the Macmillan Company New York London & Toronto 1965

6 Hunter F T, Kip A F & Irvina, J W Radioactive Tracer Studies on Arsenic Injected as Potassium Arsenite J Pharmacol Exp Therap *76* 207 1942.

7 Jung F Löslichkeit und Reaktionsweise des Arsenwasserstoffs im Blut Biochem Ztschft.. *302* 294 1939

8 Muehlberger C. W., Loevenhart A S & O Malley T S Arsine Intoxication A Case of Suspected Poisoning in the Steel Industry J Indust Hyg *10* 137 1928

9 Pernis, B & Magistretti, M A Study of the Mechanism of Acute Hemolytic Anemia from Arsine Med. Lavoro *51* 37 1960

10 Rey M European Tin and Lead Smelters Club Report on "Arsine Poisoning in the Refining of Tin Alloys" Ecole Nationale Supérieure des Mines de Paris, France 1965

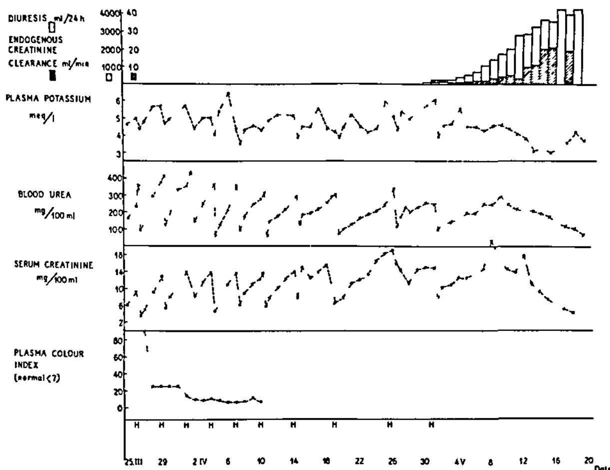


Fig 1 Graphic representation of disease course in case of severe arsine poisoning (No 5) H indicates haemodialysis

ache fever and abdominal cramps Jaundice was noticed 10–12 hours before admission the urine was dark brown

On admission the patient was febrile and strongly jaundiced but not distressed

Serum bilirubin value and serum creatinine concentration were elevated, as were serum glutamate oxalate and glutamate pyruvate transaminase and there was strong haemoglobinuria

Treatment was elevated diuresis by oral administration of fluid for several days During this period the bilirubin and transaminase values became rapidly normalised while the serum creatinine concentration did not become normal until discharge on 30 IV

On control examination on 8 XI conditions were normal

At first sight the disease picture in the 14 patients showed many points of similarity but a closer analysis of the symptomatology nevertheless shows a very variegated picture as far from all symptoms were presented by the individual patient As table I shows general symptoms such as headache malaise nausea and vomiting as well as signs of haemolysis were initially present in the majority of the patients The majority of the patients had an elevated temperature and half of them had abdominal cramp Two patients showed symptoms from the central nervous system or the peripheral nerves Ony one patient had oliguria This patient is clearly distinguished from the other patients by being far the most severely distressed (case history No 5)

Table II shows a series of the usual clinical data either from the day of admission or from one of the days immediately following With only one exception all patients had an elevated icterus index or hyperbilirubinaemia and haemoglobinuria was demonstrated in 11 of the patients by means of the benzidine test Microscopic examination of the urine showed erythrocytes in only 2 patients but in both cases following catheteriza

III

CLINICAL PICTURE AND TREATMENT IN ARSINE POISONING

by

Poul Anthonisen*) Bent Nielsen**) Kirsten Pedersen*) & Flemming Raaschou

*From the Copenhagen Kommunehospital Medical Department 3 Nephrological Section**)*
Chief Flemming Raaschou
and Glostrup Hospital Medical Department B)*
Chief Erik D Bartels

The 14 patients who were poisoned and who all survived were men aged 30–61 years As it would be exceedingly repetitious to review all the case histories only three of them will be reported These represent a mild a very severe and a moderately severe case of poisoning respectively

Patient No 2 (J No KH 2 dept 472/65) A 61 year-old man previously healthy was admitted on 26 III 1965 at 11 30 a m after having had nausea and diarrhoea over the previous 24 hours or more The macroscopic appearance of the urine was normal The patient was admitted because he had been in the same locality as the other patients for a short period of time

On admission the patient was afebrile had no jaundice and was quite unaffected

A slightly elevated value of alkaline serum phosphatase was found but a normal icterus index and a normal concentration of glutamate oxalate transaminase in the serum There was no anaemia or haemoglobinuria The serum creatinine concentration was normal

Treatment consisted of a mannitol induced rise in diuresis during the first few days The patient was discharged from hospital on 31 III in good health

Control examination on 14 IV showed normal conditions

Patient No 5 (J No KH nephrol 31/65) (Fig 1) A 60 year old man who had had repeated back pain for 5 years but had otherwise been well previously He was admitted on the 25 III 1965 at 1 45 p m after having become unwell at his work place 29 hours previously with chills and paraesthesias in the fingers In the course of the next few hours there was nausea with repeated vomiting, eventually containing fresh blood and watery brownish diarrhoea Jaundice was observed from about 20 hours before admission

On admission the patient was in distress and severely icteric As there was oliguria and rapidly increasing uraemia haemodialysis was performed as early as the next day The oliguria persisted for 40 days so that the patient had to undergo haemodialysis a total of 10 times

During hospitalization the patient had a staphylococcal sepsis which was treated with antibiotics After a period of illness lasting for one month there were attacks of diarrhoea and a mass developed under the right costal margin These conditions subsided again spontaneously in the course of about 14 days and on discharge on the 3 VII the patient was afebrile and the mass had disappeared

The patient was readmitted on 18 IX after two or three brief cholecystitis like attacks Cholecystectomy was performed on 1 X and revealed chronic cholecystitis but no stones The patient was discharged on 1 XI

After sudden chills with a rise in temperature to 39–40 C the patient was re admitted over the period 29 XI–11 XII Slight anaemia was observed a considerable rise in SR and transient pyuria and bacteriuria

During a further admission from 29 1–10 11 1966 moderate anaemia was still found Sternal marrow examination showed megaloblastic erythropoiesis and the B_{12} concentration in the serum was reduced while the folic acid concentration was normal (In view of this the results from the sternal marrow preparations from June 1965 were revised and it was now possible to see that there had been mild signs of megaloblastic erythropoiesis already at that stage) The Schilling test demonstrated strongly reduced excretion of B_{12} (3 %) and excretion was increased only slightly by the administration of intrinsic factor (6 %) A neurological examination showed discrete symptoms compatible with the diagnosis of anaemic myelopathy There was histamine refractory achlorhydria

A radioactivation analysis at this stage showed no arsenic in the patient s bone marrow

The endogenous 24 hour creatinine clearance during this last admission to hospital was still clearly reduced (54 ml/min)

Patient No 6 (J No KASGI 301211) A 34 year-old man previously healthy apart from very slight lead poisoning 18 months prior to the present case He was admitted on 25 III 1965 at 12 35 p m after a day of fatigue head

tion of the bladder The transaminases and or the lactic dehydrogenase measured showed elevated serum values in about half of the patients however this cannot be taken as a certain indication of damage to the liver parenchyma A rectal temperature of more than 37 5° C was found in about half of the patients

In 2 of the patients (Nos 2 and 12) the physical signs of poisoning were very slight or completely lacking so that the diagnosis had to be based on the anamnesis Patient No 12 had elevated serum creatinine values but this patient had hypertrophy of the prostate and presumably chronic renal disease These 2 very mild—if not doubtful—cases are included in the review because they represent the one extreme of the clinical spectrum encountered by the doctors in the departments involved

Diagnosis

It rapidly became obvious that the accumulated cases of a very uniform clinical picture must be due to poisoning at the patients place of work At the start however there was considerable uncertainty as to the agency responsible As the enterprise in question was engaged in refining various metal alloys several possibilities were pre

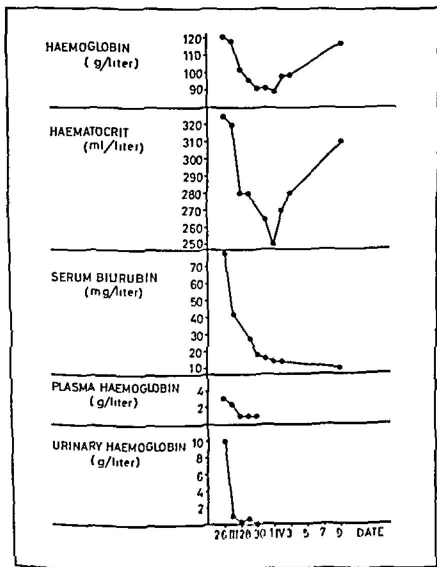


Fig [illegible] Graphic representation of data concerning haemolysis in case of moderately severe arsine poisoning (No 6)

Acta Medica Scandinavica

Supplementum 497

Atrial Septal Defect of Secundum Type in Adults

Clinical and Haemodynamic Studies of 129 Cases
Before and After Surgical Correction
Under Cardiopulmonary Bypass

By Pentti Siltanen

Published by Almqvist & Wiksell, Stockholm Sweden

Acta Medica Scandinavica

Supplementum 497

Atrial Septal Defect of Secundum Type in Adults

Clinical and Haemodynamic Studies of 129 Cases
Before and After Surgical Correction
Under Cardiopulmonary Bypass

By Pentti Siltanen

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv*, was founded in 1869 by Professor Axel Key, MD In 1901 (from volume 34) this journal was divided into a medical and a surgical section Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*

Acta Medica Scandinavica

publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German Acta Medica Scandinavica is published on a non profit basis

Subscriptions

to Acta Medica Scandinavica (two volumes of six numbers each a year) include free supplements to the current volumes During the past few years an average number of 17 supplements has been issued per annum

Subscription Rates

Per annum = two volumes
In Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Sw kr 120, incl postage
Other countries Sw kr 140, incl postage

Chief Editor

Associate Professor Birger Strandell, MD
Birger Jarlsgatan 9
S 111 45 Stockholm, Sweden

Editorial Office

Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising

Medicinska Annonsexpeditionen AB
Biblioteksgatan 11
S 111 46 Stockholm, Sweden

Subscription and Distribution

Almqvist & Wiksell
Gamla Brogatan 26, Box 62
S-101 20 Stockholm 1, Sweden

Printers

Almqvist & Wiksells Boktryckeri AB
Box 616
S 751 25 Uppsala 1, Sweden

I dedicate this work
with esteem and gratitude
to my parents
to my family
to my patients and
to my friends

Pentti Siltanen Atrial Septal Defect of Secundum Type in Adults
Acta Med Scand Suppl 497

ERRATA

Page	*Column*	*Line*	*For*	*Read*
40	Table 5 Caption	6	500	250
57	1	7	larger	smaller
92	Fig 16 Caption	9	16 0	12 3
92	Fig 16 Caption	10	4 7	3 6
93	2	26	(19 %)	(20 1 %)
93	2	28	(17 0 %)	(13 1 %)
93	2	28	40 cases	48 cases
98	2	23—24	other during the auricular systole The corresponding peaks of interatrial pressure gradient	tive component being small as a rule This suggestion constitutes a possible explana ion
127	2	5	increase	decrease
132	1	3 u	low	high

Non essential printer's errors have not been listed

FROM THE FIRST MEDICAL CLINIC AND THE THIRD SURGICAL CLINIC
UNIVERSITY CENTRAL HOSPITAL, HELSINKI AND FROM THE WIHURI
RESEARCH INSTITUTE, HELSINKI FINLAND

ATRIAL SEPTAL DEFECT OF SECUNDUM TYPE IN ADULTS

CLINICAL AND HAEMODYNAMIC STUDIES OF 129 CASES BEFORE AND AFTER SURGICAL CORRECTION UNDER CARDIOPULMONARY BYPASS

BY

PENTTI SILTANEN

ACADEMIC DISSERTATION

TO BE PRESENTED WITH THE ASSENT OF THE FACULTY OF MEDICINE OF THE UNIVERSITY OF HELSINKI FOR PUBLIC EXAMINATION IN AUDITORIUM 19 ON DECEMBER 18 1968 AT 12 O CLOCK NOON

HELSINKI 1968

Mikkeli 1968 Oy Länsi Savon Kirjapaino

ACKNOWLEDGEMENTS

The present investigation was suggested to me by my chief and teacher, Professor Pentti I Halonen M.D Head of the First Medical Clinic University Central Hospital Helsinki and of the Wihuri Research Hospital The greater part of the patients constituting my series had been preoperatively examined in one of these two establishments directed by him and in the cardiovascular laboratory of his clinic I was allowed to carry out the haemodynamic examinations involved in the follow-up studies Professor Halonen s support at the different stages of my work and his encouraging criticism during progress of this report have been extremely valuable to me and I feel the deepest gratitude towards him

The late Professor Olavi Peräsalo M.D former Chief of the Third Surgical Clinic University Central Hospital Helsinki who performed the operations on all but one of the patients in my series displayed great interest in my work and his kind encouragement has meant very much to me Professor Pekka Tala M D present Chief of the same clinic aided my work greatly by giving me the opportunity to perform the follow-up studies of the series in his clinic within a short period for which my sincerest thanks are due to him

A considerable proportion of the preoperative data used in this work are based on examinations performed with expert knowledge and experience by a number of my colleagues Without specifically stating their names I wish to acknowledge my great obligation to all of them Many are the stimulating talks which I have had with them in the course of several years and which have supplied their own essential contribution to the crystallization of the ideas presented in this report When my work was nearing its completion I had in particular the great advantage of valuable advice and positive criticism received from my friend and colleague at work, Docent Kalevi Pyörälä M.D to whom I express my appreciation of all this aid Excellent advice was further given by Docent Erkki Laustela M D in questions concerning surgical procedures and by Docent Rolf Köhler M D in those concerning the radiological part of my work

Invaluable assistance was rendered in the measurements associated with electrocardiography by Tapio Uusi-Simola M.D., and in designing and constructing the hydraulic pressure generator used in testing the pressure measurement equipment by Mr Jorma Surakka Esko Kaila Ph D gave his untiring help in the clarification of mathematical problems and decisively facilitated the arrangements connected with the calculations necessitated by my work Uljas Attila M.Sc., took care of a considerable part of the mathematical treatment of results he also designed and supplied the draftsmanship of most illustrations and the revision of the English manuscript and its partial translation are his work Timo Partanen M Sc was responsible for the statistical computer analysis of the haemodynamic findings Mr Einari Luuksela prepared part of the illustrations Miss Aino Nikupaavo M.A checked the references Many of my colleagues were helpful in gathering suitable control subjects for my studies The staff nurses and other personnel of the cardiovascular laboratory x-ray departments and bed wards spared no effort in aiding and speeding up the progress of my work Warmest thanks are due to all these persons who assisted me in various ways as well as to those nearest to me for their willing support in the course of this prolonged work

Helsinki November 1968

Pentti Siltanen

CONTENTS

I INTRODUCTION AND OBJECT OF THE STUDY

Atrial septal defect (ASD) especially its secundum type is the commonest congenital cardiac anomaly in adults It may be asymptomatic over a prolonged period, or it causes only mild symptoms in infancy and childhood The defect may occasionally even remain unrecognized until late years but in the majority of cases it results in considerable disability and often in death before middle age

Chances for repairing the defect have been created by the introduction of cardiac surgery the swift development of surgical methods has resulted in the fact that during the past 15 years surgical treatment has been applied in a continuously increasing proportion of the cases detected Accordingly ASD accounts for a considerable part of the basic cardiac surgery material in many centres For instance at the Clinic for Thoracic Surgery of the University Central Hospital Helsinki which only admits patients aged 15 years or older ASD constituted the indication for operation in 14 6 % of 1211 cardiac operations performed during the period of 16 consecutive years prior to 1967 (*Tala et al 1967*)

The success of surgery in treatment of ASD has been evaluated in numerous clinical studies On the ground of the favourable experiences gained the opinion is generally accepted that surgical treatment is usually of benefit and that it is the method of choice particularly in uncomplicated cases However there are still very few detailed follow-up studies of surgically treated cases of ASD This is particularly true as regards their haemodynamic aspects and it is moreover to be noted that such reports as can be found concern mostly only small and selected series Especially reports concerning older patients are very scarce and they are contradictory as to their conclusions It has been claimed even quite recently that most middle-aged patients with ASD would not be eligible for surgical treatment

In the series of patients subjected to surgery for ASD in our hospital, the number of individuals aged 40 years or older has approximated 20 % of the total during several years In addition, the severity of the condition has varied considerably among the older patients as well as the younger ones Therefore the material in question appears appropriate for a detailed evaluation of the clinical and haemodynamic results of surgery for ASD in adult patients

The peculiar and characteristic feature of ASD setting it apart from most other congenital heart defects is the slow progress of its clinical course which does not lead to manifestation of annoying symptoms until in adulthood often not until ages past 30—40 years Rather little is known as regards the causes responsible for this characteristic course although implication of many different factors has been suggested The age distribution of the surgical material from our hospital covers very satisfactorily those ages at which ASD seems to be prone to cause symptoms most commonly Hence it probably is a suitbale object of an analysis regarding the mechanism of deterioration

Although the clinical picture of ASD is considered to be a highly typical one it is still a fact that many cases escape proper diagnosis until the disease later takes a complicated course Methods other than recatheterization are widely used in order to discriminate cases with incomplete surgical closure of the defect although their validity has not been checked very often

The aims of the present study can be outlined as follows

1) To evaluate the clinical and haemodynamic results of open heart surgery under extracorporeal circulation in adult patients with ASD of secundum type particular attention being paid to the results obtained in the case of middle aged subjects

2) To assess the significance of clinical methods other than catheterization in the diagnosis and evaluation of ASD and as means to check on the results of surgery

3) To analyze the course of the disease in patients with ASD prior to surgery with the aim of obtaining an idea of the average natural history of the disease as encountered in the present series and to analyze the likely mechanisms of deterioration involved This implies that particular attention centres on evaluation of the right and left atrial and ventricular functions each of them by itself in terms of electrocardiographic and haemodynamic findings before and after surgery and including comparisons between the groups of middle-aged patients and of younger patients

II SURVEY OF THE LITERATURE

ANATOMY

Rokitansky (1875) divided on embryological basis the ASD into two categories the primum and secundum defects Although the understanding of the topography of these defects has since increased this classification is still the most practical one and it is commonly used The era of open heart surgery has only brought refinements in respect of different types of primum or endocardial cushion defects (Rogers and Edwards 1948 Wakai and Edwards 1956 Bedford 1960) A third type of defect with different embryology (Pernkopf 1954 Hudson 1955) has since been added namely the sinus venosus defect (synonyms high secundum defect superior marginal defect) which was first described by Wagstaffe (1868)

The *ostium secundum defect* (persistent ostium secundum) is the commonest variety of ASD which is present in about 94 % of all cases primum defects excluded (Bedford 1960) It occupies the fossa ovalis region and has a border of septal tissue at least against the atrioventricular valves (Bedford 1960) This type of defect may be limited to the fossa ovalis area alone (fossa ovalis or central defect) it may extend downward behind the Eustachian valve without any border to separate it from the opening of the inferior caval vein (inferior caval defect) or it may reach the posterior wall of the atrium (posterior defect) in which case it is often found without any separating border of septum over the openings of the pulmonary veins (pseudoanomalous pulmonary veins) These different types of secundum defect result from somewhat different developmental disturbances in the formation of the foramen ovale (Patten 1938)

Sinus venosus defect — Arrested growth of the primitive septa in the sinus venosus (Pernkopf 1954) results in sinus venosus defect This type accounts for about 6 % of the defects other than those of primum type in Bedford s (1960) material It occupies the junction of the superior caval vein and the right atrium and is of moderate size (2—3 cm in diameter) It is nearly always associated with an anomalous upper right pulmonary vein which usually opens into the superior caval vein immediately below the opening of the azygos vein and with another draining the veins of the middle lobe of the right lung into the defect at the atriocaval junction

Primum defects — Disturbed fusion of the atrioventricular endocardial cushions leads to various types of primum defect (Costa 1931) These defects are located in the anterior septum of the atria they have no separating rim of septal tissue against the atrioventricular valves and they often extend across the atrial floor into the membranous ventricular septum The mitral or both atrioventricular valves mostly present cleavage and/or hypoplasia of the septal cusp The prevalence of primum defect in adults is less than 10 % of all cases of ASD (e g Bedford 1960)

Secundum defect may appear in combination with either of the other two and in very rare instances all three types of defect are concomitant Secundum and sinus venosus defect may fuse together to form a large high defect It is obvious that haemodynamic conditions at the atrial level in the sinus venosus and secundum defects differ from those in primum defects because there is regurgitant flow through the atrioventricular valve in most cases of the latter Similarly the impairment of haemodynamics on the ventricular level in primum defects is different from that in sinus venosus and secundum defects In addition the surgical treatment of primum defects is more complicated and it has become common practice to treat the clinical features of primum

defects apart from those of the other types of ASD which on the other hand have largely similar pathophysiological consequences

The atrioventricular valves are often somewhat thickened in ASD without evidence of any rheumatic process (Davidsen 1960) but on the other hand ASD is the only congenital heart disease which is relatively often associated with rheumatic valvular lesion Especially mitral stenosis was a relatively frequent finding in earlier autopsy series it was present in 20 % of 190 cases compiled by Davidsen (1960) from the literature History of rheumatic fever was recorded in about two thirds of these cases Bedford (1960) found mitral stenosis in about 8 % of 190 cases established as ASD at surgery The reports of the prevalence of mitral stenosis seem to be mostly overestimations due to misinterpretation of degenerative but nonobstructive valvular changes Many of these changes may represent local fibroelastosis probably resulting from endocardial reaction to high blood flow rate (Okada et al 1968)

HAEMODYNAMICS

The first cardiac catheterization in ASD was performed by Brannon et al in 1945 Reports have since been published in rapidly increasing number concerning the haemodynamic findings in ASD Comprehensive reviews of the most important findings in this field and reports of personal observations have been presented e g by Dexter (1956) Soulie et al (1959) Bedford and Sellors (1960) Reindell et al (1962) Marshall et al (1962), Schrire e al (1963) and Derra et al (1965)

The arteriovenous oxygen difference is usually greater than normal and the systemic flow at rest is normal or subnormal (e g Dexter 1956 Marshall et al 1962 Schrire et al 1963 Petersson 1967) Systemic arterial oxygen saturation is usually normal while the pulmonary arterial oxygen saturation is high as a result of left-to-right shunt at atrial level and it is often so close to that of the systemic artery that calculation of the pulmonary flow by using Fick s equation is difficult or impossible During exercise the left to right shunt either remains unchanged or decreases (e g Jonsson et al 1957 Scebat et al 1957 Swan et al 1958, Davies and Gazetopoulos 1966 Petersson 1967 Strano et al 1967, Nielsen and Fabricius 1968)

With the exception of very small defects with small shunt flows the size of the defect has been found to have minor significance in determining the magnitude of the left-to-right shunt as compared to the difference between the compliances and thus to the inflow resistances of the stiff left ventricle and the more compliant right ventricle subjected to equal filling pressure (Barger et al 1948 Hull 1949 Dexter 1956) or compared to the difference between the resistances in the pulmonary and systemic circulation (Hawthorne et al 1956 Scebat et al 1957, Amorim et al 1962) or simply to the effect of the changing relationships between the diastolic filling and systolic ejection periods at various heart rates (Johnsson et al 1957) The ratio of stroke volumes of the right and left ventricle at different heart rates e g during exercise display in cases without pulmonary hypertension a degree of inverse relationship to the heart rate or oxygen consumption as can be seen from the results of Jonsson et al (1957) Scebat et al (1957) Davies and Gazetopoulos (1966) Petersson (1967) and Strano et al (1967)

In cases of small defect there is always a pressure drop of 1—5 mm Hg from the left to the right atrium but still in defects proven at surgery to be up to 3 cm in diameter there may be a gradient of several mm Hg (Cohn et al 1967), which demonstrates that the anatomical defect may be physiologically partly compensated by some mechanism At least to some extent the pressure gradient may be accounted for by the inertia of the large shunting blood volume (Levin et al 1968) The pressure gradient is often absent in cases with large defect but in fact a minute transmural pressure gradient must be present across the defect to act as determinant of the magnitude and direction of the shunt flow The direction of this gradient apparently varies in the course of the cardiac cycle as has been shown e g by Schaffer et al (1954), Braunwald et al (1956), Grosse-Brockhoff et al (1957) and Levin et al (1968) and especially in the late diastole there is often a short reversal of the shunt, which sometimes causes slight fall in arterial oxygen saturation In addition admixture of inferior caval blood to the left atrial contents has been demonstrated by angiography and by the indicator dilution

technique in about one-fifth of all secundum defects and occasionally this may be the cause of arterial desaturation leading to polycythaemia and clubbing (Lind and Wegelius 1953 Swan et al 1954 Winters et al 1967) Similar small right-to left shunts also from the superior vena cava have been shown in cases of sinus venosus defect (Swan et al 1957) Often a certain amount of systolic peak pressure gradient from the right ventricle to the pulmonary artery is found It may amount to 40 —50 mm Hg without proper anatomical pulmonary stenosis It can usually be considered a sign of relative pulmonary stenosis due to high right ventricular stroke volume (e g Schrire et al 1963) but often an artefact owing to high systolic flow rate may be concerned (Jonsson 1958)

The normal pulmonary vascular bed responds to increased output of the right ventricle by augmentation of the total cross sectional area of the resistance vessels which results in a drop of the pulmonary vascular resistance to subnormal values (Marshall et al 1959) A greater share of the increment of pulmonary flow is at first taken up by the upper lobes of both lungs and later by the right lung in this process (Dollery et al 1961) The normal physiological limits of this mechanism allow the flow through the pulmonary vascular bed to increase up to about 10 l/m² *BSA* without increase in pulmonary artery pressure (Dexter 1956) Increase of the pulmonary flow past this value results in hyperkinetic pulmonary hypertension If the total reserve of the resistance vessels is either anatomically or functionally limited owing to pulmonary arterial and arteriolar disease the pulmonary vascular resistance and also the pulmonary artery pressure will go up and obstructive pulmonary hypertension ensues Clinical experience has shown that hyperkinetic pulmonary circulation with normal pulmonary pressure and especially hyperkinetic and obstructive pulmonary hypertension may induce right ventricular failure in cases of ASD Elevated pulmonary resistance pulmonary artery pressure and right ventricular systolic pressure are associated with increased filling resistance of the right ventricle which ultimately may rise higher than that of the left ventricle and lead to reversal of the shunt

According to Dexter (1956) and Tikoff et al (1965) right ventricular failure cannot result in elevated right ventricular end-diastolic and right atrial mean pressure if the left ventricle is normal because the left ventricular end-diastolic pressure determines the level of the mean pressure in the physiologically common atrium Consequently elevated right atrial pressure is a sign of elevated left ventricular end diastolic pressure and thus denotes left ventricular failure or dysfunction In cases where the right atrial pressure is elevated findings such as slight to moderate mitral incompetence arterial hypertension and myocardial disease of the left ventricle are common (Dexter 1956 Wood 1958 Tikoff et al 1965) This contention has been questioned among others by Gibson (1958) and by Bedford and Sellors (1960) on the grounds that conditions of the kind in question and of the severity mentioned do not usually cause heart failure and clinically there remains a group in which the sole cause of heart failure appears to be progressive right ventricular failure (Schrire et al 1963) So far no closer information is available on left ventricular function in cases of ASD with elevated jugular venous and right atrial pressure before or after closure of the defect

PREVALENCE

Atrial septal defect is the commonest congenital heart disease in adults and of all congenital cardiac defects it most frequently permits survival beyond middle age The justification of this generally accepted view is illustrated e g by the following figures At 4936 consecutive autopsy examinations Fisher et al (1962) found altogether 210 cases of congenital cardiac defect The prevalence of atrial septal defect (ASD) in the age groups of less than one year 1—20 years 21—40 years and over 40 years was 23 22 35 and 37 % respectively of all congenital cardiac defects The same authors reported about 50 % prevalence of ASD among patients subjected to cardiac catheterization at an age higher than 40 in contrast to e g 17 % of 4050 catheterizations in all ages according to Derra et al (1965) Wood (1957) detected among 900 cases of congenital heart disease ASD in 18 % the proportion of persons older than 40 was 15 5 % contrasted with 3 % in ventricular septal defect 7 1 % in patent ductus arteriosus 8 % in

solitary pulmonary stenosis and 1 % in Fallot s tetralogy Similar figures of prevalence of ASD were also presented in the series of Campbell et al (1957) According to various epidemiological studies the prevalence of ASD in the living adult population has been estimated to be for instance 0 18 per thousand in Australia (Seldon et al 1962) 0 2 per thousand in Denmark (Davidsen 1960) 0 4 per thousand in Sweden (Carlgren 1959) and 0 5 per thousand in Finland (Linko et al 1963 Hakkila et al 1965) In America the population frequency of atrial septal defect has been estimated at 0 7 per thousand (Nora et al 1967) The high prevalence of ASD compared to other congenital cardiac anomalies and the increase of its relative incidence with age in the adult population is readily obvious from these data Distinct preponderance of females can be noted in most series the female-male ratio being usually less than 2 5 1

NATURAL HISTORY

The natural history of ASD is not very well known so far although several surveys of this topic can be found in the literature The older reports which relate mainly to necropsy findings have been comprehensively reviewed by Davidsen (1960), who assembled data from 190 postmortem examinations since 1811 In about 75 % of these cases cardiovascular symptoms had been present and the deaths had ensued before the age of 50 suggesting distinct overmortality of ASD as compared to corresponding general mortality figures The average age at death in the entire series was 38 2 years Campbell et al (1957) found largely similar figures in their detailed follow-up study of 100 cases of ASD namely more or less severe disabling of about 75 % of the patients before their sixth decade of life and 39 years average age at death They state as a summary of their analysis of the clinical course that the patients are actually well during the first two decades despite their large shunt enlarged heart and some vague symptoms but in the third decade the symptoms rapidly increase and in the fourth decade usually one half of the patients become seriously disabled and about one-fourth die Almost all patients in the fifth and sixth decades present symptoms most of them having developed pulmonary hypertension or congestive heart failure Besterman (1961) made a clinical study of 41 cases of pulmonary hypertension in ASD and concluded, as previously e g Dexter (1956) as well as Bedford and Sellors (1960) had done that pulmonary hypertension is rare before 20 and relatively common after 40 years of age He endorsed Dexter s (1956) statement that in ASD pulmonary hypertension is mostly an acquired complication and only rarely congenital as it is in ventricular septal defect and in patent ductus

In eight deaths in Besterman s series the average age was 39 years Bedford (1961) reports six deaths in a series of 400 cases of ASD all of them presenting pulmonary hypertension and their average age at death 40 years Wood (1962), too in his survey of the natural history of ASD gives 39 years as the average age at death in those 6—11 % of the cases which he considered by congenital mechanism to be disposed to develop pulmonary hypertension In the rest which display no pulmonary hypertension he considers the life expectancy to be relatively good and he thinks that longevity is probable for many of them However about half of the last-mentioned group have disabling symptoms especially atrial fibrillation and congestive heart failure at the age of 50 Bedford (1961) and Besterman (1961) tender similar ideas as to the natural course of ASD but they place greater weight on the significance of acquired aetiology of the pulmonary hypertension which opinion was earlier emphasized e g by Burchell (1959) and recently by Dalen et al (1967) in their long term follow-up study of 48 patients with ASD This latter study revealed that 91 % were alive of those patients who had no pulmonary hypertension at their initial catheterization ten or more years previously, against only 25 % of those with pulmonary hypertension at the time of the first examination There was one patient however whose pulmonary artery pressure had been normal at the first examination but who now presented pulmonary hypertension The authors make the comment that for the present no conclusive criteria exist for prediction of cases in which this detrimental change is likely to occur A similar observation was made earlier also by Dexter (1956) and by Burchell (1959) The prevalence of pulmonary hypertension is about 40 % in ASD after the age of 40 that is about twice that before this

age (Bedford and Sellors 1960 Craig and Selzer 1965 Rokseth 1968) but the observations of Markman et al (1965) suggest that its prevalence does not substantially increase after the age of 50 This last-mentioned phenomenon is probably one of the explanations which may account for the relatively common clinical and autoptic establishment of longeval patients with ASD indicating that uncomplicated large atrial left-to-right shunt is usually well tolerated (e g Roesler 1934 Bedford et al 1941 Burrett and White 1945 Nerard 1948, Ellis et al 1950 Coulshed and Littler 1957, Colmers 1958 Kelly 1958 Chiong 1960 Ellis et al 1960 Rodstein et al 1961 Sommer and Voudoukis 1961 Fisher et al 1962 Novack et al 1963 Adams 1965 Kuzman and Yuskis 1965) Walker et al (1956) and Storstein and Efskind (1963) point out that pulmonary hypertension in ASD is most common during the first and after the third decade of life suggesting two different aetiologies a congenital and an acquired one

About 50 % of the patients with ASD probably live longer than 40—50 years (Burrett and White 1945 Roesler 1934 Kelly 1958) but owing to slow or rapid deterioration the ten-year survival rate at middle age is only about 20 % (Daicoff et al 1967) The commonest causes of rapid deterioration are appearance of atrial fibrillation pulmonary infarction and respiratory infection (Markman et al 1965) but in frequent instances no apparent cause can be detected Slowly progressing disability may be due to rising pulmonary arterial pressure in fact several years severe disability usually precedes death in pulmonary hypertension (Besterman 1961 Dalen et al 1967) Heart failure or a condition resembling it (Wood 1962) may appear with increasing age but rarely before the age of 30 irrespective of the level of pulmonary pressure and lead to reversal of the shunt (Bedford et al 1941 Dexter 1956) Heart failure may cause mortality in ASD also in earlier infancy as Ainges and Pate (1965) have pointed out In fact heart failure is the principal cause of death in about 50 % of the cases (Davidsen 1960) or even more (Burrett and White 1945 Rodstein et al 1961) Pulmonary artery thrombosis may occur as complication of heart failure and as an aetiological factor in the acquired pulmonary hypertension typical of ASD or as a complicating disorder in pulmonary vascular disease (Bedford et al 1941 Dexter 1956 Besterman 1961) Its role appears less important as a complication in wide-age range materials than was thought earlier (e g Somerville 1964), but in aged patients it may be a noticeable cause for deterioration and death (Campbell 1957 Kelly 1958) Bacterial endocarditis is fairly uncommon in combination with solitary ASD (Hudson 1965) although in earlier autopsy series it was the cause of death in about 9 % (Davidsen 1960) Most authors agree that in numerous cases perhaps in the majority no particular cause for deterioration can be established

To sum up ASD is the commonest congenital heart disease in adults and on the average allows a longer life expectancy than any other such disease Its natural course and prognosis depend largely on appearance of pulmonary hypertension which definitely impairs the prognosis and lowers the average age at death to 40 years The condition is also serious in most cases with normal or moderately elevated pulmonary pressure but with large left-to-right shunt After the age of 50 severe disability is usually present owing to atrial fibrillation congestive heart failure and often reversal of the shunt Considerable longevity is possible in many of these cases but no possibility exists to predict which cases are of this kind

CLINICAL PROFILES

The clinical picture of ASD was first retrospectively approached in a series of autopsy cases reported by Roesler (1934) but the first detailed description of the clinical features of ASD was given by Bedford Papp and Parkinson in their now classical treatment of 1941 Accounts of clinical series have since been published by several authors and the clinical picture of ASD has become well established (Nerard 1948 Barber et al 1950 Wood 1950 Leatham and Gray 1956 Campbell et al 1957 Soulie et al 1959 Davidsen 1960) Diagnosis of ASD is normally possible clinically at the bedside and it is achieved by heeding the frequently characteristic traits in the history and suggestive features in the findings at physical radiological and electrocardiographic examinations More detailed assessment of the haemodynamic state and final confirmation of the diagnosis is attained by cardiac catheterization

History

Patients with small defects and many of those with larger uncomplicated defects are symptom-free and display good physical fitness for many years as was noted in the preceding paragraph dealing with the natural history of ASD Burrett and White (1945) have pointed out that physical examination *per se* offers little or no assistance in the diagnosis of ASD but correct clinical diagnosis can be made on the basis of long-standing mild symptoms without early cyanosis coupled with physical radiological and electrocardiographic findings indicative of primary right heart embarrassment The commonest symptom is effort intolerance due to fatigue dyspnoea or both Palpitation and syncope are fairly common precordial pain recurrent respiratory infections and haemoptysis may occur In cases of increasing pulmonary pressure tardive cyanosis appears at first only during exercise but later also at rest It may also be associated with right ventricular failure due to excessive volume load

Physical findings

The arterial pulse pressure is usually small The right ventricular impulse is diffuse and heaving The first sound is usually loud and often split The second heart sound is widely split and this is unaffected by inspiration Wide fixed splitting has been noted in various patient series in 70—100 % of the cases (Barker et al 1950 Leatham and Gray 1956, Kelly and Lyons 1958 Reindell et al 1962, Storstein and Efskind 1963 Petersson 1967, Gault et al 1968) except in pulmonary hypertension in which there is only a narrow split if any and the pulmonary component is accentuated Besterman 1961) A detailed study of the auscultatory and phonocardiographic findings in ASD was made by Leatham and Gray (1961 On the pulmonary area there is a moderate to loud systolic ejection murmur only rarely associated with a thrill, and particularly in pulmonary hypertension a clicking ejection sound occurs which is due according to the authors mentioned to dilatation and distension of the pulmonary artery Sometimes for the same reason there is a faint proto diastolic murmur of pulmonary incompetence A mid-diastolic, often high tricuspid inflow murmur is usually heard except in cases with small shunt flow and it is often preceded by a tricuspidal opening snap Owing to late closure of the pulmonary and early opening of the tricuspid valve resulting from volume overload of the right ventricle, the timing of the murmurs from tricuspid inflow and pulmonary incompetence is very similar and they may sometimes be difficult to differentiate (e g Schrire et al 1963) The defect itself usually produces no extrathoracically audible murmur although diastolic murmurs can be recorded by intracardiac phonocardiography (Wennevold 1966) Continuous murmur in the lower sternal area in cases with small atrial septal defect and with left atrial hypertension due to mitral valve disease has been desribed (Ross et al 1963) Sometimes an atrial systolic murmur is heard in presystole An extracardiac systolic murmur transmitted from the peripheral pulmonary artery and due to high pulmonary flow may sometimes be audible in the back or in the axilla (Perloff et al 1967) The jugular venous pressure may be normal but it is often slightly raised and occasionally markedly elevated The jugular venous pulse frequently shows an M-shaped pattern (Haroutounian et al 1958) or a prominent *v* wave preceded by an exaggerated *x*-descent (Reinhold 1955), both of which are helpful in diagnosis

Radiology

In earlier times the diagnosis mainly rested on radiological findings which were first described by Assman (1929) and subsequently amended by several other investigators (e g Roesler 1934 Heim de Balzac 1939 Bedford et al 1941 Burrett et al 1945 Lind and Wegelius 1953 Saltzman 1954, Campbell et al 1957 Kjellberg et al 1959 Reindell et al 1962 Arnfred 1967) The salient findings common to most reports are normal or hypoplastic left side of the heart and aorta enlarged cavities of the right heart cardiomegaly, pulmonary plethora bulging and pulsating of the pulmonary artery and its branches Davidsen (1960) stated that the heart volume correlates positively on the shunt volume and he presented a regression equation for estimating the predicted heart volume from the known size of a left-to-right

shunt A corresponding relationship was also established by Jonsson et al (1957) Davidsen recommends estimation of the predicted heart volume since by comparing this with the actual volume one may gain information on a potential complicating myocardial fault Thus radiological findings may be of great value in assessing the status of the myocardium In addition radiological examination constitutes a useful method for differential diagnosis in ASD Diagnosis of sinus venosus defects of anomalous pulmonary venous drainage and to some extent approximation of the shunt volume and pulmonary artery pressure may often be possible from the ordinary antero posterior and lateral radiograms sometimes supplemented with tomography (Keats et al 1956 Dow 1959 Reindell 1962 Fouche et al 1963) The substantial contribution of cardioangiographic methods to the diagnostics of ASD in the past calls for no further comments However their importance in ASD diagnostics has declined in the extent in which knowledge concerning the clinical picture presented by ASD has increased

Electrocardiography

Previously greater significance in diagnosis was attributed to electrocardiograms revealing right bundle branch blocks of various grades than to physical findings on account of their common occurrence in ASD (Roesler 1934 Routier et al 1940, Burrett and White 1945) Routier s series of 300 electrocardiograms in congenital heart diseases contained 22 cases with right bundle branch block and ASD was present in 20 of them It would seem that no true right bundle branch block is concerned but merely a manifestation of selective hypertrophy of the basal portions of the right ventricular wall with terminal conduction delay (Walker et al 1956 Blount et al 1957 Kjellberg et al 1959 Davies et al 1960) In extensive clinical series the prevalence of complete right bundle branch block in ASD varies from 3 % to 74 % and is mostly about 20 % The prevalence is highest in series consisting of older persons The prevalence of incomplete right bundle branch block varies from 61 to 80 % and that of signs of right ventricular hypertrophy from 21 to 67 % Atrial fibrillation is present in about 10 % of the cases almost exclusively in those older than 30 years These figures have been compiled from the series of Barber et al (1950) Kelly and Lyons (1958) Davies et al (1960) Sommer and Voudoukis (1961) Reindell et al (1962) Novack et al (1963) Derra et al (1965) Markman et al (1962) Petersson (1967) and Gault et al (1968) The severity of some electrocardiographic changes shows statistically some correlation on haemodynamic findings for instance signs of right ventricular hypertrophy are quite often associated with elevated pulmonary pressure and the incomplete or complete right bundle branch block pattern with large shunt volumes However the value of the electrocardiogram in diagnosis of pulmonary hypertension is highly limited in the individual case (Walker et al 1960 Davidsen 1960 Lee and Scherlis 1962 Schrire et al 1963 Zaver and Nadas 1965 Petersson 1967) The atrioventricular conduction time is mostly at the upper limit of normal range or slightly prolonged in sinus venosus and secundum defects and somewhat longer in primum defects The atrial electrocardiogram has been superficially considered in most reports while only Sanchez-Cascos and Deuchar (1963) so far have presented a more thorough study They found commonly signs of right atrial enlargement and sometimes left atrial enlargement and positive correlation between the length of the P wave and the magnitude of the left-to-right shunt

Electrocardiography has though not consistently (e g Davidsen 1960) proved a highly useful and reliable means for differentiation in the majority of cases of the endocardial cushion defects from ostium secundum and sinus venosus defects (Blount et al 1956 Toscano-Barboza et al 1956) on the strength of left axis deviation and early forces pointing abnormally upwards to the left (Burchell et al 1960) or terminal forces which develop counterclockwise and are directed upwards to the right (DuShane et al 1960) in the ventricular activation in primum defects

Variation of findings in different clinical groups

The clinical picture encountered in cases with obstructive *pulmonary hypertension* is often somewhat different from that ordinarily

seen in uncomplicated cases A good survey of this subject has been presented by Besterman (1961) Palpitation and often severe dyspnoea on exertion are the commonest complaints Orthopnoea is not infrequent History of recurrent respiratory infections and of congestive heart failure is elicited in about half of the cases (in 33—85 %) The deterioration is more rapid after appearance of symptoms in the obstructive than in the hyperkinetic type of pulmonary hypertension and the progression of disability in both [illegible] seriously faster than that in the common low pressure high flow variety Cyanosis is highly common in the obstructive group On auscultation pulmonary ejection sounds are heard in most cases of the obstructive type and the second sound is single or narrowly split with accentuated pulmonary component Except for a murmur of pulmonary incompetence the murmurs are mostly silent or absent The hyperkinetic variety differs from the uncomplicated one by extremely *active right ventricular lift and accentuated* pulmonary second sound The electrocardiogram often displays the right ventricular hypertrophy pattern yet not regularly The radiological findings are suggestive but they do not generally display any conclusive signs of pulmonary hypertension. Often however the lung vasculature reveals abrupt diminution in calibre at the level of the tertiary (segmental) branches of the pulmonary artery while the secondary branches still pulsate and the periphery of the lungs appears clear (e g Steiner 1958 Gay and Franch 1961)

In *primum defects* after higher mortality in the neonatal period and during infancy, the clinical course is greatly similar to that in the secundum and sinus venosus defects The incidence of pulmonary vascular disease has been claimed to be high in primum defects (e g [illegible] et al 1956 Keith et al. 1958 Wood [illegible]) but it appears not to differ essentially from that in secundum *defect if cases having* a shunt at ventricular level and individuals younger than five years are excluded (Bedford 1960 Besterman 1961 Somerville 1964) Physical examination usually elicits a systolic apical murmur which is greatly variable individually in respect of transmission and character but is mostly characteristic enough to be distinguished from pulmonary aortic and tricuspid murmurs Radiology does not contribute to the differential diagnosis of primum defects The heart volume is usually slightly larger in primum than in secundum defects (Schrire et al 1963), but this is of no diagnostic value In contrast to radiology electrocardiography is an important method in differentiation between primum and other atrial septal defects as the preceding discussion has shown.

In *sinus venosus defects* the symptoms usually appear in earlier youth (Brock and Ross 1959) and pulmonary hypertension is perhaps more prevalent (Burchell 1959) Thus in Bedford s (1960) series its incidence was twice that in ostium secundum defects and three of Besterman s (1961) four cases had obstructive pulmonary hypertension However conclusive inferences are precluded by the small number of cases reported up to date It is not impossible moreover that there is some overestimation because the size of the defect and that of the shunt are usually moderate only and many of the low pulmonary-pressure cases may not develop any symptoms whereby *they escape diagnosis In the roent*genograms ampullary dilatation of the inferior part of the superior vena cava is often characteristic and the abnormal pulmonary veins are often visible or may be anticipated by reason of exceptional vascularity of the right hilus (Dow 1959 Brock and Ross 1959) Cardiac catheterization reveals elevated oxygen content of the superior caval blood several centimetres cephalad to the right atrium and deviation of the catheter tip into openings of anomalous veins often confirms the diagnosis Impaction of the catheter on the left side through the defect does not usually occur by the standard route but succeeds in frequent instances higher up at the atriocaval junction when the catheter is advanced in downward direction

Persons over 40 years of age often present an atypical and bizarre clinical picture in cases of ASD (Sommer and Voudoukis 1961) owing *to increasing complications of ASD and other* superimposed complicating diseases The typical features of longeval cases were discussed in the preceding paragraphs It remains to add that apparently no essential differences exist in clinical course between the different anatomical types in adults In recapitulation, the characteristic features in patients of advanced adult age are rapid increase in prevalence of pulmonary hypertension between 30 and 50 years increasing prevalence after 40 years of

atrial fibrillation and heart failure (Wood 1962) of pulmonary thrombosis (Dexter 1956 Campbell et al 1957, Kelly and Lyons 1958) and of angina of effort (Rodstein et al 1961) The largest heart volumes are encountered in patients older than 40 years (Davidsen 1960 Reindell et al 1962) In the electrocardiograms the prevalence of right bundle branch block (Coulshed and Littler 1957 Kelly and Lyons 1958) and the duration of the QRS complex (Burch and DePasquale 1967) increase with age The primary S-T segment and T-wave changes in the chest leads too become more common with increasing age (Davidsen 1960 Novack et al 1963 Markman et al 1965) as well as the right ventricular hypertrophy pattern (Novack et al 1963 Gault et al 1968) great or bifid P wave and retarded atrioventricular conduction (Campbell et al 1957 Cohn et al 1967)

SURGICAL TREATMENT

In the early 1950s a number of surgical procedures were developed for closure of ASD in experimental animals and in man Of the closed techniques the atrio septopexy of Bailey et al (1952) was the first successful approach in human surgery for ASD it was soon followed by the atrial well technique of Gross et al (1953) and the circumferential occlusion of Søndergaard (1954) and of Bjork et al (1954) Open heart surgery permitting closure of the defect under direct visual control was first applied clinically by Lewis and Taufic (1952) who performed correction of ASD under general moderate hypothermia and temporary inflow occlusion Approximately contemporaneously Swan reported a series of successful operations with the aid of hypothermia (Swan 1953 Blount et al 1953 1954) The first operation involving open correction of human ASD with total heart-lung bypass and extracorporeal circulation was carried out by Gibbon in 1954 The closed methods have largely been abandoned since but they are still being used in some places

At present there is no controversy concerning the use of open procedures in correction of primum defects and in these cases extracorporeal circulation is generally preferred in most centres In respect of secundum defect opinions are divided as to which one of the two open techniques should be preferred In many centres most of the operations in uncomplicated cases are performed partly for practical reasons with general hypothermia and inflow occlusion often combined with selective coronary perfusion (Bjork et al 1960 Storstein and Efskind 1963) On the other hand there are centres where extracorporeal circulation is invariably applied in cases of ASD The inquiry instituted by the Committee of Cardiovascular Surgery of the American College of Chest Physicians 1962 yielded from several European and American centres accounts of altogether 2145 operations for secundum-type ASD Of these 18 5 % were performed by closed methods 25 3 % with the aid of hypothermia and 56 2 % with extracorporeal circulation Of about 5000 operations assembled at random from the reports of several teams to be found in earlier and more recent literature about two-fifths had been made with hypothermia likewise approximately two-fifths with extracorporeal circulation and about one-fifth by a closed method i e by the well technique or by circumclusion

The mortality figures and the incidence of complications seem to depend rather more on the experience of the surgical team on the nature of the lesion and on presence of complicating diseases than on the type of operation Thus in the accounts elicited by the said inquiry the operative mortality varied between 3 and 4 % and the incidence of atrioventricular conduction disturbances from 2 to 5 7 % in secundum defects regardless of the type of the operation while the respective figures in the primum defect group were 26 % and 16 % In pulmonary venous transpositions too the operative mortality exceeds that of uncomplicated secundum defects (Mustard 1960 Derra et al 1965) The Committee states that occasional necessity of prolonged operation time is the prime reason for the common agreement on extracorporeal circulation as the method of choice in repair

Pulmonary hypertension clearly increases the risk of operation as can be seen from the variation of the mortality figures between 40 and 71 % in a number of earlier series (McGoon et al 1959 Liddle et al 1960 Cooley et al 1966) However Besterman (1961) reported an operative mortality of 25 % in ASD with hyperkinetic pulmonary hypertension Sellers et al (1966) one of 11 % and Gault et al

(1968) state that pulmonary hypertension does not significantly raise the operative mortality past the overall one in ASD According to Derra et al (1965) the mortality figures are significantly affected by elevation of the pulmonary vascular resistance past 800 dynes sec cm^{-5}

Congestive heart failure has been reported to imply substantially increased operative mortality (Cooley et al 1966)

Advanced age seems to entail higher operative or postoperative hospital mortality and frequency of complications Thus, the mortality of persons under 20 years is usually less than 1 % (e g Bedford 1961 Petersson 1967) while 6—11 % has been reported for patients over 40 (Bowles et al 1966 Daicoff et al 1967, Petersson 1967 Gault et al 1968) and 18—21 % for those over 50 (Cooley et al 1966 Gault et al 1966) Even mortalities as high as 40 % for patients over 45 years have been stated (Wolf et al 1968) On the other hand Derra et al (1965) think that age has no significance as regards the operative mortality in ASD Comparison of different clinical series is always difficult owing to their unequal composition Particularly in series consisting of elderly patients risks of greatly different level may be introduced by the different proportions of severely disabled individuals

Surgical treatment is generally considered to be indicated if the pulmonary-systemic flow ratio is 1 5—2 0 or higher quite often regardless of presence or absence of symptoms (e g Swan 1963 Wood 1957, Derra et al 1965 Cohn et al 1967) A warrant of correct diagnosis alone has been considered sufficient for surgical intervention by some authors (e g Mustard 1962) The age between 5 and 15 years has been considered most appropriate for surgery in ASD (Efskind et al 1959 Mark 1963 Petersson 1967) Surgery is contraindicated according to most authors when a balanced or predominantly right to left shunt is present with or without pulmonary hypertension but recent reports exist of good results also in selected cases of this kind if a special surgical technique is applied (Cohn et al 1967)

RESULTS OF SURGERY

The best method in testing the results of surgical correction of ASD known so far is cardiac catheterization This method often combined with various dye gaseous and thermal indicator dilution techniques has been used since the very start of operative treatment of ASD in the centres involved as a check on successful elimination of the shunt The prevalence of residual shunt after closure of secundum and sinus venosus defects was generally 10—25 % in the 1950s and less than 20 % in the 1960s As determined by routine catheterization methods the frequency figures were 7 % 6 7 % 16 7 % 7 % 13 % and 0 % according to Loogen et al (1961) Reindell et al (1962), Schrire et al (1963), Sellers et al (1966) Arnfred (1967) and Petersson (1967) respectively radioactive gas indicator studies have yielded prevalence figures of 7 % (Cohn et al 1967) and 13 % (Gault et al 1968) The results are not dependent on the technique employed if open operations are considered by themselves In contrast closed techniques seem to result more often in a residual shunt (e g Carlgren 1961 Sondergaard 1962 Sellers et al 1966)

Although there are numerous reports concerning series of catheter-proven surgically treated cases of ASD fairly little information can be found on detailed clinical and haemodynamic preoperative and postoperative evaluation of ASD cases as e g Cohn et al (1967) and Petersson (1967) have recently pointed out Most generally the postoperative haemodynamic data reported are confined to statement of presence or absence of shunt and of the pressure levels in the right ventricle and pulmonary artery Distinct decrease of these pressures and of pulmonary vascular resistance after successful operation was already reported by Blount et al (1953 1954) This beneficial circulatory change has since been treated by many authors who have found that the pulmonary pressure is lowered in most cases including those with normal preoperative values (Kirklin et al 1956 Winchell and Bashour 1958) The pulmonary vascular resistance also decreases in most instances with complicating hypertension (Beck et al 1960 Cohn et al 1967) although in some cases very little if any decrease is noted (Loogen et al 1961 Besterman 1961)

The postoperative haemodynamic condition in ASD with obstructive pulmonary hypertension was more closely investigated by Beck et al (1960) They found postoperatively at

rest the pulmonary pressure and pulmonary vascular resistance to be reduced by about half of their preoperative value but they noted, during exercise in recumbent position marked increase in pulmonary vascular resistance which was less marked if the patient breathed pure oxygen These results they interpreted to be evidence of increased vasomotor tone in the pulmonary arterioles and they considered the transmural pulmonary arterial pressure to be one of its determinants The pulmonary artery wedge pressure was measured during exercise in six cases (41 5 years mean age) in two of which a rise greater than normal was established which may possibly be suggestive of left ventricular abnormality Unfortunately the types of defect were not stated in the cases involved In cases with preoperative congestive heart failure in older adults the possibility has been pointed out that after closure of the defect excessive rise of the left atrial pressure ensues as a result of preexisting left ventricular failure and which leads to pulmonary venous congestion or pulmonary oedema (Wolf et al. 1968)

The commonly encountered systolic pressure gradient between the right ventricle and pulmonary artery has been found to disappear in most instances (Kay and Zimmerman 1958 Loogen et al 1961 Reindell et al 1962) even when it amounted to 50 mm Hg (Schrire et al 1963)

By the time the present examinations were completed (February 1967) no reports on more detailed clinical electrocardiographic and radiological evaluation combined with thorough haemodynamic evaluation of the results of surgery in any more extensive series of ASD had appeared in the literature Petersson (1967) has very recently contributed a long way towards rectification of this shortcoming by his comprehensive study on the preoperative and postoperative condition in 107 cases of uncomplicated secundum defect corrected under hypothermia He found uniform postoperative decrease of all the pressures in the right heart invariably normal wedge pressures and normal haemodynamic response during exercise except for features of hypokinetic circulation especially in children and older adults However only 16 of his 57 adult cases were 40 years of age or older and the patients presented exceptionally good preoperative condition For instance there was one single case of atrial fibrillation none of congestive heart failure and there were only six patients in functional class III and none in class IV In the same year Daicoff et al (1967) reported on 155 patients aged 45 years or older who had been operated on with excellent results using cardiopulmonary by-pass Of his series 13 % were bed-ridden (class IV) while 25 % belonged to class III Unfortunately he gives no closer information on the postoperative haemodynamic data The same applies to the recent reports by Gault et al (1968) and by Rokseth (1968) in which only the main preoperative and postoperative flow and pressure data of patients older than 40 years are reviewed

Reports on the influence of surgery on functional capacity in terms of the N Y H A classification (New York Heart Association 1964) have been presented by Arnfred (1967) Petersson (1967) Daicoff et al (1967) and Gault et al (1968) It seems that impairment of physical capacity is exceptional the rule being improvement by one class or more The unfavourable results in elderly patients reported by Wolf et al (1968) seem to be exceedingly rare The physical fitness was preoperatively and postoperatively measured by ergometry in the studies of Reindell et al (1962) and of Petersson (1967) Distinct slow improvement was observed in the former series while there was no change in the latter

As regards the commonly reported unequivocal regression of physical signs postoperatively only occasionally closer details can be found Exceptions are the auscultatory and phonocardiographic findings which have been throughly analyzed by several authors (e g Eisenberg and Hultgren 1959 Loogen et al 1961 Reindell et al 1962 Aygen and Braunwald 1962 Petersson 1967 Wennewold 1967) Abnormal heart sounds and diastolic murmurs disappeared in all cases except in residual pulmonary hypertension in which the protodiastolic murmur of pulmonary incompetence and systolic ejection sounds may persist Systolic pulmonary murmur disappears on the average in half of the cases and is reduced in strength in the rest Fixed split of the second sound becomes narrow and changing during the first six postoperative months in the majority of cases constituting an indication of haemodynamic normality Persistence of the split

second sound is found to be commoner in cases with more incomplete regression of the prolonged QRS complex in the electrocardiogram (Loogen et al 1961)

The electrocardiographic postoperative changes have been a frequent subject of analysis Walker et al (1956) found that the mean frontal QRS axis moves towards the left and the R/S ratio in the V_1 lead decreases if the operation is successful Leftward shift of the mean QRS frontal axis shortening of the QRS complex and diminution of the R or R deflection in lead V_1 have been considered to possess some value in assessment of the result of surgery the last-mentioned change in particular occurs rather regularly after correction of ASD (e g Dreifus et al 1959 Davies et al 1960 Reindell et al 1962 Lee and Scherlis 1962 Arnfred 1967 Petersson 1967) The changes in voltage usually take place slowly over a period of months while shortening of QRS and its axis shift ensue rapidly on operation (Dreifus 1959 Lee and Scherlis 1962 Reindell et al 1962) Virtually complete normalization of the electrocardiogram does not exclude the possibility of a small residual shunt but it is strongly in favour of normal haemodynamics (Loogen et al 1961) On the other hand a bundle branch block pattern may persist even though there is no shunt

The radiological evaluation of results of surgery has recently been reviewed and studied by Arnfred (1967) He found diminution o heart volume by about 20 % in all cases preoperative heart enlargement Diminu of the heart shadow was already reporte Blount et al (1954) and subsequently numerous other authors (e g Walker e 1956 Swan et al 1959 Mahoney et al 1 The heart volume rapidly diminishes p operatively and usually displays some inc again after termination of postoperative (Reindell et al 1962) In the series of Lo et al (1961) no change of the heart vol ensued in 30 % of the cases and of the pat aged 20 years or older 59 % presented changed heart volume The authors were able to explain this finding on the basi anatomical myocardial changes alone Arr (1967), too and Swan et al (1959) before point out that in older persons the hea larger relative to the shunt volume, remains larger postoperatively compared young patients The size of the pulmo artery often diminishes (Reindell et al Petersson 1967) yet not consistently (Bl et al 1954, Arnfred 1967) and the regre of pulmonary vascular markings is even i uncertain (Blount et al 1954 Arnfred 1! The characteristic pulsation of the pulmo artery usually disappears and the aortic sations clearly increase as can be observe fluoroscopy and kymography (Reindell e 1962)

III MATERIAL, AND GENERAL OUTLINE OF THE PRESENT STUDY

The present material consists of 129 patients subjected to operation for ASD of secundum of sinus venosus type during the period 1960—1966 at the Third Surgical Clinic The operations were carried out with the aid of extracorporeal circulation and heart lung bypass All but one of the operations were performed by one and the same surgeon (the late Professor Olavi Perasalo M D) mainly with assistance by the same team

The preoperative right heart catheterizations were performed at the First Medical Clinic in 86 cases at the Third Medical Clinic in five at the Pediatric Clinic in two at the Hospital of the Wihuri Research Institute in 32 at the Kuopio Central Hospital in three and at Katharinenhospital Stuttgart Western Germany in one case Thirteen of the preoperative right heart catheterizations and 105 of the preoperative clinical electrocardiographic and radiological evalutions were personally performed by the author Furthermore at follow-up all the preoperative records tracings and roentgenograms were reexamined in detail by the author who also made all the postoperative evalutions with the exception of the cardiac catheterization in one case of residual shunt All the postoperative examinations were carried out at the Cardiovascular Laboratory of the First Medical Clinic and at the Third Surgical Clinic during the period July 1966—February 1967

COLLECTION OF MATERIAL

Altogether 136 cases of ASD primum defects excluded were operated upon from December 1960 to July 1967 In one case originally included in the series it was necessary to postpone the operation until the autumn of 1967 The indications for operation which were applied were pulmonary flow/systemic flow ratio higher than 1 5 1 or progressing pulmonary hypertension Balanced or right to-left shunt with or without pulmonary hypertension was considered to constitute a contraindication to surgery Congestive heart failure or advanced age in themselves were not considered to be factors imposing limitations on operability

Two out of 136 patients could not be contacted for follow-up because they had emigrated These two one woman aged 22 and a man aged 27 years had both had a large left-to-right shunt with only slight preoperative symptoms and no pulmonary hypertension the course of operation and postoperative convalescence period were uneventful and the primary result was good in both cases These patients have not been seen after discharge from the hospital Five other patients have died three of them at or immediately after the operation (one male and two female patients aged 49 28 and 34 years) while one female patient (39 years) succumbed three months postoperatively to an unrelated disease (postappendicitic intestinal occlusion) and one further female patient (38 years) who presented Lutembacher's syndrome died three years after surgery owing to progression of the disease 129 patients thus remained for the follow-up study and they constitute the present material

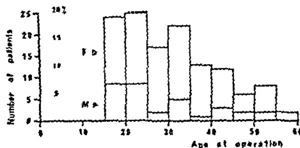


Fig 1 Number of patients of the present series (F — Female M — Male) in different age groups at the time of operation

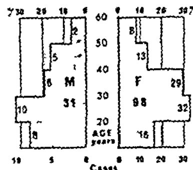


Fig 2 Age distribution of the male and female patients comprised in the present series compared to the approximate distribution of the entire population of Finland (dotted lines)

DISTRIBUTION BY SEX, AGE AND RESIDENCE

Table 1 and the histogram Fig 1 show the figures relating to age at the time of operation and to sex All the patients were over 14 years of age 101 of them were younger than 40 (young age group) and 28 were 40 years or older (old age group) The highest age in the series is 60 years The female male ratio in the entire series is 3 2 1 and there is no substantial difference between different age groups in this respect The mean age of the entire material is 31 2 ± 11 0 years No significant difference in mean age exists between the female and male subseries The groups of patients under 40 years and of 40 years or older have mean ages which are about 21 years apart

The percentile age composition of the series [illegible] sented in Fig 2 compared to that of [illegible] population of the country It is to b[illegible] he age groups above the age of 40 years are [illegible] ly smaller in the present series than in [illegible] ormal population

The pa[illegible]en[illegible] ng to urban population constitute 36 [illegible] series contrasted to 42 % urban popula[illegible] n the whole country 29 % of he pat[illegible] were residents of Helsinki or its vicinity [illegible] came from Southern Finland 41 % from Centr[illegible] Finland and 12 % from Northern Finla[illegible] The corresponding proportions reflecting the distribution of population among the same areas are in rough figures 10 40 35 and 15 [illegible] respectively

GENERAL DESIGN OF THE STUDY

The patients of the series were invited to present themselves for follow-up examination in the order in which they had been operated on They were examined in the order in which they obeyed the invitation The patients were admitted for at least one day or for three days if subjected to catheterization The subjects younger than 40 years subsequent to the one who brought the number of catheterized examinees of this age group up to 30 were examined at the hospital s out-patient department All but two of the patients aged 40 years or older (28 in number) were catheterized but in one of them only superior caval angiography was feasible In respect of the younger subjects catheterization was invariably included in the schedule until 30 such catheterizations had been completed these catheterizations thus conform to the succession in which the patients arrived at the hospital the examination being omitted only if the facilities were occupied by a previously admitted patient or by one of the group over 40 years This procedure was scrupulously adhered to in order that the 30 young subjects for haemodynamic studies might be randomly selected Right heart catheterizations were thus made in altogether 58 follow-up cases also during exercise in 53 of them and invariably combined with a dye dilution test The cases not subjected to catheterization were tested for residual shunt by the peripheral dye injection technique In addition to clinical

examinations the following were performed in every instance electrocardiography phonocardiography determination of physical working capacity by the bicycle ergometer and roentgenological examination The patients had all been catheterized prior to operation in most cases less than one year previously Preoperative phonocardiography existed in only 15 cases and preoperative determination of physical working capacity in 38 The selection for this latter test had been entirely unrelated to the patient s condition

The follow-up periods average 29 6 ± 15 0 months (range 5—57 months) for the entire series the corresponding figures for the group of patients aged 40 years or older and for the younger patients are 25 3 ± 13 7 (range 5—57) and 30 8 ± 15 1 (range 7—72) respectively The follow-up period was less than six months in one case only and less than one year in seven

CONTROL GROUPS

Since the aims set for the present study included no comparison of treated and untreated cases no control series proper was required and the patients themselves furnished the reference data For the evaluation of some electrocardiographic measurements the tracings obtained from 50 completely healthy members of the police force were examined A similar purpose in connection with certain radiological measurements was served by the clinical findings of 100 patients with apparently healthy cardiovascular system as judged on the basis of their clinical findings who had been admitted to the hospital for short periods As an aid in evaluating the physical working capacity data the working capacity and heart volume of 26 healthy nurses were determined In addition to this normal material data relating to 44 healthy men were available who had previously been examined in similar manner by Frick (1961) In order to facilitate the treatment of the results of catheterization five healthy individuals presenting functional innocent murmur were subjected to right heart catheterization at rest and during exercise Twelve further cases which had yielded technically acceptable tracings and normal findings at routine right heart catheterization previously performed either at the Wihuri Research Institute or at the First Medical Clinic mostly by the author were adjoined to the normal series for use in closer analysis of the catheter data

COMMENTS

The present material differs in respect of its composition from most previously reported series as regards its primary selection on the basis of age Accordingly a fairly high proportion of the present patients belong to the age group in which symptoms and even disability are known to be prevalent Consider-

Table 1 Sex and age distribution of the present series

Patient group	N	Age in years $\bar{x}$	Age in years S D
Age < 40	101	26 7	7 2
Females	77	27 5	7 1
Males	24	24 1	6 6
Age ≧ 40	28	47 8	5 7
Females	21	48 0	5 8
Males	7	47 0	5 3
All ages	129	31 2	11 0
Females	98	31 9	11 4
Males	31	29 2	12 0

ing this selection procedure and the manner in which patients are conventionally selected for surgery the present age distribution can be said to approximate that of earlier similar series The preponderance of young age groups in the percentile age distribution of the present series as compared with the normal population may be due to possible over-mortality in patients aged 40 years or older

The sex distribution (female male 3 2 1) differs markedly from that in most series covering all ages previously reported female-male rations varying in the range of 1 5 1 to 2 5 1 (e g Campbell et al 1957 Wood 1957 Soulie et al 1959 Davidsen 1960 Bedford 1961 Zaver and Nadas 1965 Cohn et al 1967, Petersson 1967) Although similar figures have been stated concerning series of older patients e g Sommer and Voudoukis 1961 Cooley e al 1966 Gault et al 1968) or even lower ratios (Kelly and Lyons 1958) markedly higher ratios ranging from 2 9 1 to 4 1 are the rule in series comprising mainly adults or elderly individuals (Bedford et al 1941 Rodstein et al 1961 Novack et al 1963 Markman et al 1965 Daicoff et al 1967) This might suggest a somewhat different natural history of ASD in patients of different sex

The present distribution by residence displays some prepoderance of urban population compared to the national population figures in particular inhabitants of the city of Helsinki are over-represented as a natural consequence of the hospital s admission policy, this being a regional central hospital Some under-representation of other areas in the southern part of the country can be noted caused by the existence of another active centre of cardiac surgery (in Turku) during the period in question On the whole however the present material may be considered representative of the whole country as regards residential distribution

The selection of patients for postoperative haemodynamic studies among those younger than 40 years can be considered to have been random in strict contrast to the group of patients aged 40 years or older in which all subjects were catheterized if possible Both samples are therefore representative of their respective group and consequently mutually comparable

SYNOPSIS

Of 136 patients who had been subjected to operation for ASD of secundum type or of sinus venosus type under cardiopulmonary bypass during the period 1960—1966 129 were available for follow-up study This series comprises 98 females and 31 males The age of 28 patients was 40 years or higher This age group is distinctly under-represented as compared with the normal population probably as a result of overmortality at ages over 40 years in ASD The series includes residents of all parts of the country The female male ratio is somewhat high 3 1 1, compared to those in other younger series reported which may suggest some difference in the natural course of ASD in men and women

All the subjects had been preoperatively catheterized and postoperative catheterization was performed in 58 cases in 54 of them also during exercise Phonocardiography and determination of physical working capacity had been made preoperatively in 15 and 38 cases respectively and postoperatively in every case Preoperative and postoperative clinical and radiological data are available in regard of every patient Dye dilution curves were preoperatively obtained in nearly all instances and postoperatively in every case

Data intended to aid the evaluation of the results including records and tracings of miscellaneous kind were obtained from a total of more than 200 healthy individuals of different ages and of both sexes

The follow-up period averaged 29.6 ± 15.0 months being shorter than one year in seven cases only

IV SURGERY

All but one of the operations involved in the present series were carried out by the late Professor Olavi Perasalo In 85 % of all cases the surgical intervention took place later than January 1963 when the author joined the surgical team as cardiologist assuming responsibility for the patients immediate preoperative and postoperative management and control of their cardiovascular state during operation

The period of continuous monitoring regularly covered the day of operation only the exceptions consisting of cases in which complications were present and in which this period was extended are specifically stated below Whenever respiratory failure was to be expected particularly if the patient had low pulmonary function values or obstructive pulmonary hypertension Engstroms respirator was applied for several postoperative days The conventional principles of postoperative care were otherwise followed

PERFUSION TECHNIQUE

The operations were invariably performed under perfusion mostly combined with moderate hypothermia The perfusion technique employed has been described in detail before (Siltanen et al 1966) In 37 % of the 136 cases constituting the original surgical series haemodilution technique was applied using a disposable-bag bubble oxygenator In the earlier cases of the series the apparatus used was a disc oxygenator and whole blood was used for perfusion The impression was gained on the basis of clinical indications that the perfusion period and immediate postoperative course were both more uneventful in the dilution technique group but no statistically significant difference in incidence of complications exists between both group

SURGICAL TECHNIQUE

Consideration of technical aspects is not thought to be indicated here apart from a few remarks Median sternotomy was invariably used in the early operations and subsequently in selected cases but anterior right thoracotomy through the fourth intercostal space was the procedure in the majority of the cases reported Since 1964 the operating surgeon exercised particular care at atriotomy to mind the artery of the sinoatrial node even though this node could rarely be identified The caval cannulations were made in conventional manner by retrograde route from the right atrium the exceptions to this in which more peripheral venous cannulation was used were reoperations in two cases of diversion of the inferior vena cava into the left atrium and two operations in cases presenting sinus venosus defect Closure of the defect was most often in 81 % of the cases accomplished by direct suturation with interrupted stitches A Teflon patch was applied in the rest Simultaneous pulmonary valvotomy was performed in one case Eight cases with pulmonary venous anomalies necessitated special measures those methods were applied in them which have been reviewed e g by Barclay (1960)

FINDINGS

Central secundum defect was established in 108 of the cases included in the follow-up series (in 84 %) The defect was less than 3 cm

in diameter in about one-third (31 %) of the cases all the rest presenting rather large defects In two cases associated smaller fenestration of the septum was noted

Inferior caval secundum defect was encountered in eight instances (6 2 %)

Posterior secundum defect of moderate size was seen in five cases and in another five cases a large posterior defect was present which situation is often referred to as pseudo-transposition of pulmonary veins

Sinus venosus defect was found in three instances (2 3 %) together with one fatality (specified farther below) this amounts to 3 1 % incidence of this type of defect in the original surgical series of 136 cases This kind of defect was invariably associated with anomalous pulmonary venous drainage

Anomalous pulmonary venous drainage was seen in a total of eight cases (6.2 %) These include three instances in which all the veins of the right lung opened into the right atrium or the upper atriocaval junction (sinus venosus) four with the upper lobe veins only and one with the vein of the right upper lobe opening high into the superior vena cava

Valvular and other abnormalities — In one case the defect was associated with a presumably rheumatic affection of the mitral valve causing slight incompetence Counting also the previously mentioned case with mitral stenosis (Lutembacher's syndrome) in which death ensued before follow-up study rheumatic mitral valve involvement was thus found in only two out of 136 consecutive operations (1 5 %) in the surgical series One case presented mild valvular pulmonary stenosis and another comparatively free pulmonary regurgitation due to a deformed and calcified pulmonary valve which had presumably been destroyed by bacterial endocarditis Left superior vena cava was encountered in four case

COMPLICATIONS

Non-fatal surgical complication occurred in three cases Two cases presented diversion of the inferior vena cava to the left side of the atrial septum Correction was later accomplished by reoperation in both cases in one of these there was obstructive pulmonary hypertension while the other patient had had severe congestive heart failure prior to the first operation No difficulties were encountered at the reoperations The third case was one in which closure of sinus venosus defect resulted in narrowing of the superior vena cava later leading to total obstruction as evidenced by follow-up angiography This patient still refuses to submit to reoperation

Fatal surgical complications occurred in three cases all three being among the 27 earliest operations of the series The subsequent part of the surgical series 109 consecutive operations was thus managed without one single fatality In one of the fatal cases (male 49 years) the cause of death was a dissecting haematoma of the abdominal aorta which was ruptured during operation The origin of the haematoma was an intimal lesion usured by the metal cannula in the left femoral artery The cause of death in the second case (female 28 years) was a cerebral complication due to air embolism For the third fatality (female 34 years) accidental rupture of the superior vena cava at the end of the operation in a case of sinus venosus defect was responsible The defect in all three cases was a large left-to-right shunt without complications and the cause of death was entirely unrelated to the patient s condition

Non-surgical complications were not fatal in any case of the series

The postoperative complications not related to surgical technique which were recorded in the follow-up series of 129 patients are listed in Table 2 Altogether 65 complications were encountered in 41 cases (32 % of the total series) which amounts to concomitance of complications at a ratio of 1 58 1 in the entire material The incidence of various arrhythmias was 27 % (of 129 cases) and they account for 63 % of all complications In 27 patients (27 %) of the young age group 38 complications were noted equivalent to complications per ca e at 1 41 1 Various postoperative arrhythmias occurred in this age group in about 20 % The old age group had 14 patients (50 %) with altogether 27 complications equivalent to the ratio of 1 93 1 The prevalence of arrhythmias

Table 2 Number of different postoperative complications not related to surgical technique in patients younger than 40 years and in patients aged 40 years or older

Complication	Age < 40 (27 out of 101 cases)		Age ≧ 40 (14 out of 28 cases)	
	Number of complications	Per cent of age group	Number of complications	Per cent of age group
Cardiac				
Atrial fibrillation *	5	5 %		28 6 %
Atrial flutter	2	2 %	2	7 1 %
Severe sinus tachycardia	2	2 %	—	
Atrial tachycardia	1	1 %	3	10 7 %
Nodal rhythm	6	6 %	—	
Coronary nodal rhythm	1	1 %	1	3 6 %
Sino-atrial block	1	1 %	1	3 6 %
Atrioventricular block	1	1 %	—	
Right bundle branch block	—		1	3 6 %
Frequent ventr premat beats	3	3 %	2	7 1 %
Ventricular tachycardia	1	1 %	—	
Ventricular fibrillation	1	1 %	—	
Pericarditis	3	3 %	—	
Congestive heart failure	3	3 %	1	3 6 %
Embolism				
Arterial	—		1	3 6 %
Venous	3	3 %	2	7 1 %
Respiratory	5	5 %	3	10 7 %
Other				
Psychosis	—		1	3 6 %
Abdominal	—		1	3 6 %

* In addition atrial fibrillation was present already before the operation in 11 cases of the older age group and in one patient younger than 40 years

in this old age group amounted to 54 % No correlation existed between arrhythmia frequency and heart volume magnitude of the shunt or level of pulmonary of arterial pressure nor could any sex preponderance be seen The incidence of postoperative arrhythmias was probably slightly lower after 1964 but without any statistically significant difference There was one single case of atrioventricular block presumably due to stretching of tissues which subsided completely in two days

Altogether 16 non-surgical complications during operation (minor arrhythmias excluded) occurred in 13 cases namely temporary sinoatrial block twice eight atrioventricular blocks of various grades atrial fibrillation three times supraventricular tachycardia once and ventricular fibrillation once all of which disappeared within a few minutes and one case with obstructive pulmonary hypertension in which severe ventilatory failure necessitated treatment with buffer solutions Air embolism occurred in one male patient resulting in temporary hemiparesis but subsiding completely without any neurological sequelae in two weeks Thus the frequency of air embolism was 2 cases in the entire surgical series of 136 consecutive operations

A late postoperative febrile period ocurred in five cases which was considered to be postpericardiotomy syndrome in three of them Atypical lymphocytes appeared in the blood in two cases In them Paul Bunnel s reaction was not positive but one case had slightly elevated cytomegalovirus antibody titre Altogether five of the 34 cases of ASD of secundum type operated on during the period August 1965 to June 1966 presented more or less distinct postopera tive rise of cytomegalovirus antibody titre without any other signs of cytomegalovirus infection

COMMENTS

A feature common to most surgical series is sharp decline of mortality and of the frequency of complications after an initial period of practising the particular kind of surgery This is also apparent in the present series of 136 operations in which the mortality of the last 109 operations was nil A similar decrease is noted in the frequency of complications which were few in number considering the mean age of the series and some reports stating high complication frequency in elderly patients (Wolf et al 1968) The prevalence of complications is also low as compared to the results of Daicoff et al (1967) who found complications other than arrhythmias in 32 % of 155 patients aged 45 years or older and to those of Cohn et al (1967) who report complications in 50 % of their series covering all ages half of them other than arrhythmias In the present material complications occurred in 32 % of the cases and the percentage of complications other than arrhythmias was only 18 % It is thought that this favourable result was partly brought about by the policy of scrupulous care in preparing the patient for operation by respiratory and [illegible]her exercises and reestablishment of medication during at least ten preoperative days [illegible] early or prophylactic postoperative applicati[illegible]f assisted ventilation in cases with potential or [illegible]mpending ventilatory failure and by very ear[illegible] m[illegible]bilization after surgery

Supraventricular [illegible]rrhythmias are highly common in connectio[illegible]th open heart surgery (Popper et al 1962 [illegible] et al 1967) In ASD special disposition to su[illegible]aventricular arrhythmias is present which in[illegible]ses with increasing age as several authors have stated (Davidsen 1960 Wood 1962 Somerville 1965) and this is also evident in the present series It is not surprising therefore that operative correction of ASD is frequently accompanied by arrhythmias which Petersson (1967) for instance noted in 44 % of his series of operations performed under hypothermia In the series of Cohn et al (1967) of operations with cardiopulmonary bypass the incidence of arrhythmia was lower Its occurrence amounted to 32 % of all patients but it was only 20 % in young subjects con trasted to 54 % in older patients Taking into account the 11 cases which presented preoperative atrial fibrillation all but two of the patients in the old age group of the present series were in a rhythm other than sinus rhythm for a transient or prolonged period and atrial fibrillation was present in 68 % This is consistent with the observations of Popper et al (1962) who elicited linear increase of the incidence of postoperative arrhythmias with increasing age They state that no other precipitating factors related to the patient s condition are discernible, a fact which is also apparent in the present series They did not observe any evidence of higher incidence of arrhythmias after hypothermia either although there are reports concerning e g experimental evidence of arrhythmias and associated myocardial lesions after hypothermia and inflow occlusion (Sarajas 1961)

Hurt and Bates (1958) observed atrial fibrillation after thoracotomy in 45 % if the pericardium had been opened and in 25 % if it had not been opened While they could not find any reason for this Cohen and Pastor (1957) thought that it was attributable to vagal reflexes and anoxia The experimental and clinical observations of James (1961 1963) suggested that shift to some ectopic or other ab normal rhythm would be facilitated by depression of the sinus node dominance owing to occlusion of the sinus artery or to inflammation invasive from the pericardium This reasoning and the earlier observation that the sinus artery traverses in about 69 % of human beings the anterolateral wall of the right atrium (Halonen 1938) which usually is subjected to atriotomy instigated us in the present series from 1964 onwards to particularly careful avoidance of the sinus node or artery during surgery and undelayed steroid therapy was instituted in addition to antiarrhythmic meas

ures in all cases of postoperative arrhythmia We gained the impression that the subsequent incidence of arrhythmias was lower and their duration more limited than before, but this impression is not borne out by any statistically significant differences Recently James and his associates have clearly documented the promoting effect which sinus node trauma associated with open heart surgery has on arrhythmias (Tung et al 1967) The non spectacular effect elicited with prophylactic measures against sinus node lesion in the present series seems to suggest predominance of some other precipitating factors for instance advanced age Finally it should be emphasized that the sole instance of serious arrhythmia in the present series concerned a female patient aged 38 years with uncomplicated ASD and only moderately enlarged heart who had presumably recently gone through myocarditis

Psychotic episodes after open heart surgery have been reported mainly in older individuals (extensively reviewed by Hazán 1966) One instance of psychosis in a woman aged 52 years adds weight to the common clinical experience that anxiety and slight to moderate psychic disturbances at least are not infrequent during the immediate postoperative period and that they may be a likely factor precipitating arrhythmias In some more extensive open-heart surgery series psychosis prevalences of about 3 % have been reported but less pronounced delirious states may be encountered in up to 60 % of the cases

A syndrome resembling mononucleosis following open-heart surgery was first reported from our hospital by Paloheimo and Halonen (1965) while Kääriäinen et al (1966) recorded increased titre of complement-fixing cytomegalovirus antibodies in association with a similar syndrome in a patient subjected to VSD operation in this hospital 19 cases of postoperative subclinical cytomegalovirus infection which occurred in a total of 63 open-heart operations including the cases encountered in the present series are reported by Paloheimo et al (1968) The prevalence of subclinical cytomegalovirus infection was about 15 % in the series of 34 ASD cases figures of the same order have subsequently been found by the same team

SYNOPSIS

In the original surgical series of 136 consecutive operations using cardiopulmonary bypass three deaths occurred but the mortality was nil in the last 109 operations The non-fatal surgical complications which occurred were caval deformation in three cases in two of which reoperation was performed The majority of the non surgical complications (63 %) were arrhythmias such complications were more than twice as frequent in the group of patients aged 40 years or older as in the younger group Their causality has been discussed and apart from the promoting effect of age no positive precipitating factors can be pointed out other than the operation itself

V MEDICAL HISTORY AND PHYSICAL FINDINGS

METHODS

Conventional clinical methods were employed in eliciting the *medical history* of the patients, instead of standardized techniques which are especially applied in epidemiological studies The data were all gathered at the time of the follow up examination missing information was added at comparison with the history recorded preoperatively subject to corroboration by the patient The ages at which various symptoms appeared were recorded to the closest five years or half decade Merely the presence or absence of the symptom was taken into account no attention being paid to its degree of severity as a rule Only in respect of evaluation of functional capacity the severity of the most strongly limiting symptoms was noted following the principles laid down by the Criteria Committee of the New York Heart Association (1964) The N Y H A criteria for classifying patients into four functional classes according *to their physical capacity are generally known* In the present work they were interpreted and additional criteria were used to supplement them e g in regard of heart failure as has been suggested by the American Medical Association s Committee on Medical Rating of Physical Impairment (1960) This implies that subjects with heart failure not refractory to therapy belong to Class III while those with therapy resistant heart failure are referred to Class IV

Palpation was performed by conventional clinical methods The scale from 0 to 3 was employed in recording the findings 0 — Normal finding 1 — Slight, 2 — Moderate and 3 — Marked increase of he impulse

F *scultation* the usual technique was employed i n scle.s room and during basal conditions both in supine nd left lateral recumbent position. It was performed du ng quiet respiration over several respiratory cy le and at the end of a deep inspirium with and with ut Valsalva s manoeuvre Throughout the study with the exception of a small fraction of the preoperative examinations which was not personally made by the author the stethoscope employed was the Rapaport Sprague binaural acoustic stethoscope (Sanborn 1952) with three interchangeable bells and two diaphragms of these the $1\frac{1}{4}$ inch bell and a diaphragm $1\frac{1}{2}$ in in diameter intended for adults, were used A binary scoring was employed to indicate the loudness of the heart sounds (0 — normal 1 — loud) for the intensity of murmurs the well known six point scale of Freeman and Levir (1933) was used The widely used graphical metho of Segall (1933) was applied for the purpose of re cording the quality of murmurs. The components sounds and murmurs were selectively interprete

The *jugular venous pressure* was evaluated wit the sternal angle for reference point. Heights of 5 c or more above the sternal angle were considered t be abnormal. Except for some occasional electr recordings made with light beam guided equipmer (Atlas 4 Atlas-Werke Germany) the *jugular venou pulse* was recorded by graphic approximation i accordance with visual assessment. The *arterial bloo pressure* was measured with the aid of a mercur sphygmomanometer with 14 cm × 40 cm cuff A ordinary cuff was used in a small minority of th preoperative measurements. The measurements wer performed in recumbent position, the manomete reading for the diastolic pressure being made at th moment when the Korotkoff sounds weakened

Comments on methods — It goes without sayin that cooperative attitude of the patient is a matte of primary importance in history taking but exce sive desire to cooperate may make the patient adm non existing symptoms or exaggerate existing one In interindividual comparisons the reliability of infor mation gained by history taking is usually highl limited The interrogations evaluated in this wor were all performed by the author in person Th main sources of error which have to be minded ir clude differences in individual perception threshol of pain and discomfort, failure of memory semant and verbal misinterpretations, and in patients wit congenital disease the lack of experience of the cor dition serving as reference standard The first these aspects can be statistically controlled in seri which are extensive enough. The next two sources error may perhaps be significantly reduced in im portance in smaller series if the interrogation conducted in the conversational manner which characteristic of the clinical method of history takin as contrasted to standardized questions without ap propriate interpretation of the replies received (c Wood 1956) and if the information is dated in term of large units of time (half or full decades) Th last mentioned error is avoidable to some extent b taking the preoperative history once again after th operation, when the alleviation or disappearance o symptoms from which the patient has suffered sinc infancy has given him some idea of the true stan dard of reference

The methodic errors incurred in evaluating the palpation and auscultation findings were not subjected to any systematic check. It is common experience in clinical work that the reproducibility of the scores obtained by the methods applied here is rather satisfactory with one and the same observer as well as with different observers The phonocardiographic tracings made at follow up showed good agreement with the auscultatory findings. The width of the split in the second heart sound estimated on the basis of auscultation agreed within ± 10 milliseconds with the value elicited from phonocardiograms when the split did not exceed 30 msec Considerably greater errors were noted in cases with larger split, but even then changes by 10 msec in either direction could be distinguished Less than 10 % of the third fourth and ejection sounds escaped notice at auscultation in likeness with Heikkila s (1967) findings

The errors inherent in estimation of the systolic and diastolic blood pressures by Korotkoff's indirect auscultatory method have been discussed in detail by Karvonen et al (1964) Their studies, and numerous other investigations too reveal that the size of the cuff has an important bearing on the accuracy of the estimate Smaller differences between the directly and indirectly measured values are noted when a cuff of larger dimensions than most commercially available types is used This fact carries particular weight when diastolic blood pressures in the neighbourhood of 100 mm Hg are concerned the value which was arbitrarily chosen in the present study to represent the upper limit of normal range Indirect blood pressure measurements simultaneous with catheterization were made in a fraction of the present series their results showed good agreement with the directly measured diastolic pressures.

Assessment of functional capacity according to the N Y H.A. classification meets in practice with some problems, which require more precisely formulated criteria The improved criteria of the A.M.A Committee which were applied in this study seem to be conducive to higher accuracy in rating

MEDICAL HISTORY

Heredity

Findings — In altogether 17 cases (13 2 %) of the present series ASD or some other congenital heart lesion occurred in the patient s family in one or several persons other than the patient himself No attempts were made in this study to subject the patient s relatives to systematic investigation Out of the 17 cases ten were such in which the presence of anomaly in the relatives was positive while it was probable in seven Five cases concerned a parent or child of the patient and in five one or several sibs were involved The present series itself includes two sisters and one female patient s brother has been subjected to surgery after the gathering of material for this series was discontinued Five of the pedigrees display multiple penetration also various digital anomalies were present in two of them Anomalies of the fingers were also elicited in one further family in which so far no further case of congenital heart anomaly has been diagnosed

Comments on heredity — It has been shown in several investigations that hereditary factors contribute to the aetiology of ASD Such families have been described in which ASD occurred in two or several consecutive generations (e g Zuckerman et al 1962) This suggests that the defect may be inherited as an autosomal dominant characteristic in some families although usually its inheritance seems to be multifactorical Campbell and Polani (1961) and recently Nora et al (1967) have presented evidence suggesting inheritance of ASD in both recessive and dominant fashion The latter authors instituted a comparison of the families of 100 patients with ASD of secundum type 32 of these families had one member other than the patient himself with ASD or some other cardiac anomaly

Other diseases

Findings — In the present series history of rheumatic fever was recorded in 14 cases (11 %) of recurrent tonsillitis in 42 (33 %) of prolonged more severe febrile episodes in 14 (11 %) of recurrent pneumonia in 13 (10 %) and of pulmonary tuberculosis or of pleurisy considered to be tuberculotic in 8 cases (6 %) The cases were evenly distributed between the young and old age groups of patients

Comments on the findings concerning other diseases — The figures relating to tuberculosis and to rheumatic fever are no higher than would be expected on the basis of the corresponding data of an unselected population Prior to the 1950 s usually less than four new cases of pulmonary tuberculosis or tuberculotic pleurisy were found per one thousand population Before the second world war (in the 1930 s) in the Finnish Defence Forces for instance there were annually 15 new cases of rheumatic fever per 1000 men and in the 1940 s

only four per 1000 (Somer and Frick 1965) From these figures approximate prevalences can be derived for an arbitrary population of 30 or 40 years age which are found to be on the same level as the figures obtained in the present series which thus are not indicative of any higher incidences in connection with ASD Nerard (1948) presumed the incidence of tuberculosis to be higher among patients with ASD than among the population at large but her contention was not confirmed by any subsequent series The figure found in the present study, though exclusively based on anamnestic data is not in favour of any higher prevalence either That rheumatic affections would be prevalent in ASD is an old surmise based on the establishment of valvular lesions at autopsy in series of early times The figures in question are undoubtedly excessively high as has been pointed out before It is true however that as a rule patients with rheumatic valvular heart disease have a history of rheumatic fever in less than half of the cases whereby the clinical impression is created that the incidence of rheumatic fever may have been somewhat high in the present series Of the two patients in the series who presented rheumatic mitral involvement (only one of them included in the follow-up series) only one had a history of rheumatic fever systolic murmur suggestive of mitral regurgitation occurred in one single case among those 14 whose history contained rheumatic fever

The group of 13 patients with a history of recurrent pneumonia contains only one individual older than 40 years Only four patients out of 13 presented a pulmonary vascular resistance index higher than 300 dynes sec/cm[4]

Detection

The principal indications which led to discovery of the cardiac defects were murmur and cardiomegaly or other abnormality of cardiac silhouette in the radiogram Table 3 shows in what connections such observations were made in the cases of the present series Thus murmur was an incidental finding in 87 cases (67 %) while it was established in only 29 cases (22 %) at specific examination on account of existing symptoms Abnormal heart contour in the x-ray was incidentally observed in 62 cases (48 %) and at examination due to symptoms in 36 (28 %) It is seen that highest in screening efficiency as regards murmur were health examinations at school which contributed 28 % of the cases and in respect of abnormal heart contour the mass miniature x-ray examinations with 22 % of the present cases The average age at which murmur was observed is 15 9 years (S.D 11 5 range 0—50 years) in 57 % of the cases this age was less than 16 years Abnormal heart contour was detected at the average age of 21 4 years (S.D 12 3, range 2—58 years) with 29 % of the discoveries before the age of 16 The examinations resulting in specific diagnosis and therapy were initiated at a mean age of 28 2 years (S.D 12 3 range 3—59 years) only 12 % of the cases being such in which this occurred before the age of 16 The inducement to such examinations was *murmur radiological cardiac abnormality and* existing symptoms in 18 %, 22 % and 47 % respectively

Comments on the mode of detection — The age at which the murmurs and radiological abnormalities of the heart were discovered and the age at which the defect was diagnosed are both high compared to the series presented by Davidsen (1960) of whose cases the majority was diagnosed prior to 16 years of age The age selection of the present series offers one obvious explanation to account for this difference The late detection of the defects may also reflect the circumstance that the practitioners were not too familiar with the clinical manifestations of this particular anomaly prior to the 1960s Furthermore it seems to be a feature characteristic of ASD that owing to late appearance of symptoms the patients are late in seeking medical assistance Although the leading signs had been discovered in childhood in more than half of the cases the final examinations resulting in correct diagnosis and in surgical treatment were only commenced at adult age in the majority the inducement responsible for such examinations had then been appearance of distressing symptoms in half of the cases as contrasted to one-fourth of the cases in childhood

Mass miniature x ray examination constitutes a highly appropriate screening device for ASD (Hakkila et al 1965 Pietila et al 1968) nowa days the percentage of cases coming for opera tion for this defect which were singled out by this method even exceeds 22 %

Table 3 Distribution of 129 cases of ASD according to the connection in which *murmur and abnormal heart shadow were first detected*

Detection of murmur		
At health examination		57
— at birth	3	
— before school age	7	
— at school	34	
— at seeking employment	4	
— during pregnancy	4	
— other	5	
At examination because of cardiac anomaly in radiogram		13
At examination because of symptoms		29
At examination because of other diseases		30
		129
Detection of cardiac anomaly in radiogram		
At mass miniature x-ray examination		29
At other x-ray health examination		11
At examination because of murmur		31
At examination because of symptoms		36
At examination because of other diseases		22
		129

Symptoms

The *preoperative symptomatology* was mainly studied in terms of age distribution in the present series For practical purposes some of the symptoms were arbitrarily classified according to three groups reflecting the haemodynamic state (1) Symptoms probably related to reduced systemic or peripheral circulation, such as fatigue fainting and cold extremities (2) Symptoms probably related to congestion of pulmonary circulation such as dyspnoea on exertion paroxysmal nocturnal dyspnoea and orthopnoea (3) Symptoms probably related to systemic congestion such as oedema and nycturia Further anamnestic data which have been taken into account are symptoms such as cyanosis which reflect venous admixture of the blood anginous and non-specific chest pain syndromes palpitation related to hyperactive heart arrhythmias and symptoms related to the nervous system such as headache and dizziness

Fig 3 is a compilation showing the preoperative and postoperative frequencies of the most important symptoms (number and percentage of cases) the figure also graphically displays the distribution of each preoperative symptom according to the patients age at its appearance (in five-year age groups clearly salient modes being marked) and in respect of postoperative occurrence the distribution between the groups of patients younger than 40 years and those aged 40 or older (in numbers of cases and in percent of total) The commonest symptoms were dyspnoea on exertion (in 76 % of the cases preoperatively) palpitation (in 71 %) various dysrhythmias (in 66 %) fatigue (in 62 %) and various kinds of chest pain (in 62 %) all of which display a bimodal distribution as regards their age of appearance with higher frequency during the first two decades of the patients life The second mode is seen at later age and it is seen between the ages of 15 and 30 years in dysrhythmias other than atrial fibrillation which has unimodal character between 30 and 55 years The frequency of dyspnoea on exertion increases fairly linearly with increasing age but another mode can be discerned between the ages of 30 and 40 years Its course is rather closely duplicated by the shallow unimodal distribution of paroxysmal nocturnal dyspnoea which was preoperatively experienced by 21 % of the pa-

tients this symptom made its appearance at ages between 10 and 50 years with a maximum at about 30 years The histogram representing the appearance of palpitation is largely similar to that of fatigue both displaying accumulation of cases in the first decade

The course in terms of age of symptoms of varying degree of severity and probably related to congestion of the pulmonary circulation is more or less closely reproduced in that of congestive symptoms of the systemic circulation Oedema which was preoperatively reported in 21 % of the cases shows a bimodal histogram with one mode at about 35 years and another close to 50 years of age Nycturia (not included in the figure) occurred preoperatively in 16 % and presented a similar age distribution

Fatigue was preoperatively present in 62 % and fainting (not included in the figure) in 27 % of the cases Both are symptoms which usually distress the affected patients ever since early life although their prevalence increases in later years The symptom of cold extremities which is an extremely common complaint of these patients and occurred preoperatively in 79 % of the cases also displayed a similar age distribution

Of the symptoms recorded as chest pain the majority were non specific of their character most often described as pricking or stabbing sensation sometimes as smarting or dull ache and usually associated with minor dysrhythmias The preoperative incidence of this type of chest pain was 50 % with bimodal age distribution having its maximum frequency of appearance in the first two decades of age and a separate mode between 15 and 30 years in likeness with paroxysmal dysrhythmias It is followed by anginous pains which appeared at maximum frequency close to the age of 40 yet with detached occurrence even at early ages Intolerance of cold weather which is a complaint characteristic of coronary patients was stated in 27 % (not included in the figure) its distribution according to age at the time of its appearance approximates the histograms of both pain syndromes shown in the figure

The patients functional capacity deteriorated with increasing age 71 % of them entering the second functional class (FC2) This occurred bimodally with a maximum in the first decade of life and after substained progression a second mode between the ages of 25 and 35 years Subsequently one-fifth (22 %) of the patients entered class FC3 between the ages of 30 and 50 years while ultimately three patients impaired between 40 and 55 years to the level of FC4 53 % of the patients presenting FC3 and all those with FC4 derangement were 40 years old or older

Chronic bronchitis and coughing on exertion were fairly common before surgery occurring in 15 % and 22 % respectively Haemoptysis had occurred once in six cases and recurrently in two All these symptoms mostly appeared during the first decade of life their later appearance showing bimodal age distribution like those of oedema and nycturia

Transient cyanosis was reported in 54 cases (42 %) most often in association with exertion coughing laughing crying and sometimes during infections but there were no cases of its occurrence during rest It had appeared in childhood in most instances but another mode between the ages of 35 and 40 years is formed by eight cases of tardive cyanosis

Complaints of dizziness and headache before surgery both amount to 26 % of the cases The tendency of both kinds of distress was to appear in the early decades of life

The development of growth and particularly that of weight had been retarded before puberty in 33 % Poor health in infancy and childhood was reported in 21 % Arterial embolism had occurred in four cases two of them such in which the diagnosis was likely yet not proven Two of the four patients were younger than 40 years

The *effect of surgery* on the symptoms was a striking one as a rule On the whole the prevalence of various symptoms at follow-up in the entire series was 15 % or less in regard of most symptoms it was less than 10 % Exceptions to this were cold extremities with a residual incidence of 27 % (preoperatively 79 %), and headache the incidence of which was not substantially changed (preoperatively 26 % postoperatively 21 %) Dyspnoea on exertion was postoperatively present in 11 subjects younger than 40 years and in eight of those aged 40 years or older paroxysmal nocturnal dyspnoea only in three subjects of the latter and one of the former group and orthopnoea in six three of either group

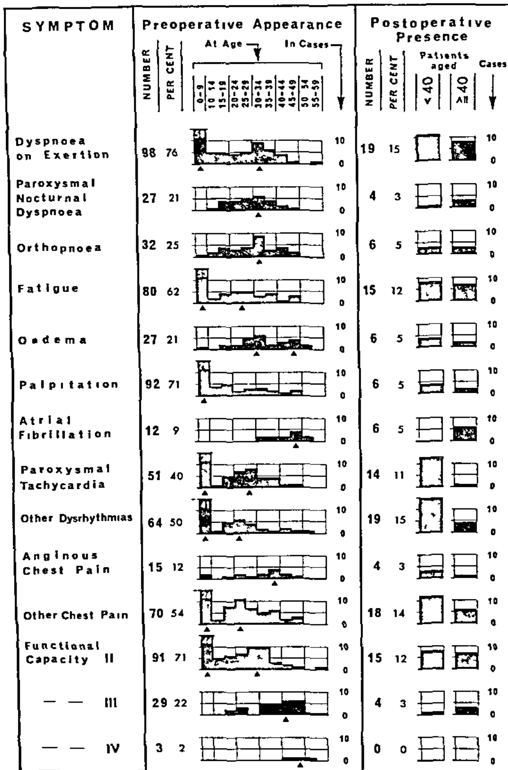


Fig 3 Time of appearance before the operation of some principal symptoms and their frequency before and after operation The histograms in the central column display the numbers of cases in which each symptom appeared during different decades of life with black arrowheads indicating salient modes In the column on the right the prevalence of each symptom after the operation in terms of number of cases is indicated by the dark pillars separately for the young age group and the old age group In both columns the lean numerals state the frequency of each symptom in percent of the entire series

The following occurrences of symptoms deserve to be mentioned in addition to those presented in Fig 3 fainting one case nycturia 5% intolerance of cold 6% bronchitis 9% coughing 3% haemoptysis one case and vertigo 5% One male patient, aged 53 years had a renal arterial embolism during the follow-up examinations following an unsuccessful attempt to convert his atrial fibrillation to sinus rhythm. In the same patient arterial embolism had also preoperatively occurred in his left leg One further male patient aged 55 years with atrial fibrillation had a cerebral embolism one year postoperatively from which he recovered without sequelae

There are some exceptional instances in which new symptoms appeared postoperatively Thus four of the seven postoperative cases of nycturia represent persistent previous symptoms in the other three cases all of them older than 40 years it appeared after surgery although all signs and symptoms of congestive failure had disappeared No renal function studies were made in these cases Further exceptions

Table 4 Functional capacity according to N Y.H.A., before and after closure of ASD in 101 patients younger than 40 years (a), 28 patients aged 40 years or older (b) and 129 patients of both age groups (c)

a) Age < 40

		Before operation				
		I	II	III	IV	Total
After operation	I	35	51	6	—	92
	II	—	3	5	—	8
	III	—	—	1	—	1
	IV	—	—	—	—	—
Total		35	54	12	—	101

b) Age ≧ 40

		Before operation				
		I	II	III	IV	Total
After operation	I	2	6	9	1	18
	II	—	1	4	2	7
	III	1	1	1	—	3
	IV	—	—	—	—	—
Total		3	8	14	3	28

c) All patients

		Before operation				
		I	II	III	IV	Total
After operation	I	37	57	15	1	110
	II	—	4	9	2	15
	III	1	1	2	—	4
	IV	—	—	—	—	—
Total		38	62	26	3	129

are headache dizziness and non-specific chest pain these symptoms were postoperative in origin in 9 out of 28 5 out of 6 and 9 out of 18 follow-up cases respectively About half of these subjects were older than 40 years

Table 4 illustrates the effect of surgery on functional capacity In the entire series all the patients who were in FC4 before the operation showed improvement the same is true for nearly all those in FC3 and for three-quarters of the patients in FC2 The percentage of patients in FC1 was 29 % preoperatively and 85 % postoperatively The corresponding figures for the subjects younger than 40 years are 35 % and 91 % and for those aged 40 years or more 11 % and 64 % respectively Impairment of functional capacity ensued in two cases only In one of them a female patient aged 42 years the preoperative FC1 condition changed to FC3 owing to disabling occlusion of the superior vena cava as a complication of surgery In the other case concerning a man aged 41 years FC2 deteriorated to FC3 because even preoperatively present effort angina was postoperatively exacerbated although the haemodynamic burden of the right heart had been greatly relieved Another two cases showed FC3 both before and after surgery One of these patients a man aged 38 years had a large residual shunt and pulmonary hypertension The other patient was a woman aged 55 years with recurrent paroxysms of atrial fibrillation with high ventricular rate In four cases FC2 remained unchanged after surgery The cause responsible for this was dyspnoea in two cases pain in the scar in one and fatigue in one Both cases with poorer postoperative than preoperative functional capacity and two of the six patients with unchanged functional capacity belonged to the group of patients aged 40 years or older

Thus, in the old age group improvement of functional capacity by one or several N Y H.A classes was noted in 21 out of 25 patients preoperatively belonging to FC2 FC3 or FC4 that is in 84 % In the younger age group 62 out of 66 such patients that is 94 % presented similar improvement Even so eleven patients did not improve any further than to the level of FC2 Of them six were members of the old age group The principal limiting factor was dyspnoea in six cases (four of them aged 40 years or older) and fatigue in five cases (two of them 40 years or older)

Preoperatively 36 patients were receiving digitalis After surgery 29 patients were under digitalis and 5 under chinidine medication

Comments on symptoms — The scope of this study does not call for any more detailed analysis of the preoperative symptoms recorded in the present series but some pertinent comments are indicated

Many of the symptoms considered display somewhat high prevalence compared to certain previously reported series In many instances the patient did not complain of a given symptom but became aware of its former presence after the operation when he had gained a new reference standard for the symptom free state Accordingly, the figures are somewhat influenced by the procedure which was followed in taking the subjects histories It should be noted however that exclusively such symptoms were taken into account for which it was possible to establish their time of appearance or which were otherwise indisputably real There is no way to estimate what effect the patients postoperative positive attitude may have exerted but it is not thought to have played any significant role On the contrary evidence is presented in Chapter IX to the effect that the patients rather tend to overestimate their preoperative capacity and slightly underestimate its postoperative level

A feature of particular interest is the high prevalence of dysrhythmias noted in the present series A certain degree of unstable heart rhythm seems to be a common characteristic of secundum-type ASD already at an early time in the first decades of life In the present series this was evident in the form of minor arrhythmias in 40 % of the patients during the first or second decade Various dysrhythmias appeared later in another 16 % The proportion of more serious dysrhythmias increased in the later decades The second mode in the histogram of Other dysrhythmias is mainly made up of frequent ectopic beats and short bursts of tachycardia (as far as such can be identified by means of history-taking) and it is located at 20—25 years paroxysmal tachycardia has its second mode at a slightly higher age and marked occurrence of atrial fibrillation follows in the neighbourhood of 50 years Appearance of dysrhythmia or its conversion to another more distressing type was usually interpreted by the patient as a significant deterioration of

his condition In ASD secundum dysrhythmias seem to play a role largely similar to that reported by Somerville (1965) in respect of ASD primum except for the fact that serious arrhythmias appear to be exceedingly rare in secundum defects Atrial fibrillation was invariably associated with deterioration as Wood (1962) has previously pointed out but it was not very often involved in the deterioration coincident with increasing dysrhythmias In the extensive combined series of Zaver and Nadas (1965) dysrhythmias were found in 20 % and the age of their onset averaged 26 2 years

The bimodal late occurrence of symptoms probably related to systemic congestion (oedema and nycturia) may perhaps be due to the heterogeneity of the population in question As has been said before, it is claimed that about 40 % of the patients over 40 years of age have pulmonary hypertension which probably does not substantially increase in frequency after the age of 50 years These figures are in accordance with the findings in the present series (Chapter XI) The frequently cited opinion was also mentioned before according to which patients with ASD complicated by pulmonary vascular disease deteriorate earlier and succumb to their disease at an average age of 40 while in patients with uncomplicated major shunt deterioration if any occurs at fairly high age Such composition of the population of two different components would account for the said feature in the age histogram In the present series pulmonary vascular resistance was abnormally high in only 2 but less than 100 dynes sec cm^{-5} in 3 out of seven cases who presented oedema at the age of 30—40 years whereas among nine patients aged 40—50 years abnormally high values were encountered in four instances and a value lower than 100 dynes sec cm^{-5} only once These findings are thus not in support of progressive pulmonary hypertension being the determinant of early deterioration It should also be noted that the patients with oedema in the young age group had a significantly smaller heart volume in addition to which there were no electrocardiographic differences between them and the older patients either The occurrence of oedema suggesting congestive heart failure presents no correlation on any of the parameters recorded in this study However, such lack of correlation between symptoms suggesting systemic congestion and haemodynamic findings is not uncommon in clinical practice

Fatigue cold extremities and fainting though generally considered to be rather non-specific symptoms would seem to possess some kind of specific significance in the present material

Table 5 Impulse of right ventricle (RV), pulmonary artery (PA) and pulmonary valve closure (PVCl), and auscultatory loudness of pulmonary component of the second sound (II P) in incipient pulmonary vascular disease (IPVD pulmonary vascular resistance index 251—500 dynes sec cm^{-5}) hyperkinetic pulmonary hypertension (HPH peak systolic PA pressure ≧ 50, and pulmonary vascular resistance index < 500 dynes sec cm^{-5}) and obstructive pulmonary hypertension (OPH pulmonary vascular resistance index > 500 dynes sec cm^{-5})

		Grade of impulse															
		By palpation												By ausc	
Group of patients	*N*	RV 0	RV 1	RV 2	RV 3	PA 0	PA 1	PA 2	PA 3	PVCl 0	PVCl 1	PVCl 2	PVCl 3	II P 0	II P 1
IPVD	10	1	4	5	—	8	2	—	—	8	1	1	—	7	3
HPH	2	—	1	1	—	1	1	—	—	1	1	—	—	2	—
OPH	3	—	—	2	1	2	1	—	—	—	1	2	—	1	2
Others	114	6	61	44	3	75	37	2	—	82	33	—	—	107	7
Total	129	7	66	52	4	86	41	2	—	91	35	3	—	117	12

judging on the basis of the marked reduction of these symptoms after surgery The theory that they might be associated with low systemic flow is opposed by the finding that the systemic flow index (Cardiac Index) was 3 7 l/min/m² *BSA (S.D 1 3)* in those who complained of such symptoms while patients free of them had an even lower index 3 5 l/min/m² *BSA* (S D 1.2) There is no statistically significant difference between the two means A similar pattern was found in the systemic arteriovenous oxygen difference which is a perhaps still more sensitive indicator of hypokinetic circulation Davidsen (1960) too states that syncope which he found in 17 % had no correlation on haemodynamic findings Syncope was most often associated with elevation to upright position or with standing both of which are known to produce orthostatic hypotensive reactions The prevalence of fainting in ASD might be a sign of deficient capacity of orthostatic adjustment in this condition According to Papp (1958) fainting in ASD is related to arrhythmias No such connection was evident in the present series

PHYSICAL FINDINGS

General

The *constitution* was normal in the entire series as a rule Of the 129 patients 22 (17 %) were classified as gracile 8 (6 %) as stout and the remaining 99 (77 %) as ordinary

Thoracic deformities were rather common A left-side or bilateral anterior thoracic bulge voussure cardiaque was noted in 52 patients (40 %) Pectus carinatum was present in three patients six had pectus excavatum two had thoracic scoliosis and normal thoracic cyphosis was absent in two Thus altogether one half of the patients presented abnormalities of the thoracic cage No systematic search for lesser bone anomalies involving vertebrae etc was made

Digital anomalies were present in three cases as has been mentioned before In all three the anomalies consisted of short phalanges and anomalous distal phalanges

Pink cheeks characteristic of ASD were preoperatively observed in 57 patients (44 %) The colour subsided after surgery in eight cases only and was accordingly postoperatively present in 49 cases (38 %) Its prevalence was much higher in the older group of patients after operation (in 61 %) but not before the operation (in 39 %)

Central cyanosis was preoperatively established in two cases only It persisted after surgery in one case with chronic bronchitis and with pulmonary vascular resistance index 351 dynes sec/cm⁵ No clubbing of fingers was found

Peripheral cyanosis was seen in 11 cases ten of them belonging to the old age group It persisted after surgery in two older and one younger patient

Blood pressures

Jugular venous pressure was considered to be elevated in 14 cases most of them patients of the old age group Hepatojugular reflux was observed in 12 cases the *v* wave was early in four and three patients had enlarged liver In the patients with normal or nearly normal jugular venous pressure exaggerated *x* through and M-shaped jugular venous pulse contour were common and usually clearly visible

Arterial pressure — The systolic pressure varied in a wide range from 100 to 210 mm Hg The range of diastolic pressure was 60—130 mm Hg it was preoperatively 100 mm Hg or higher in 11 cases ten of them older than 40 years and two of them males Diastolic pressures of 100 105 110 and 130 mm Hg were noted in 6 3 1 and 1 cases respectively Six of these without exception older than 40 years still presented 100 mm Hg or higher diastolic pressure after surgery in addition to them nine further cases were found whose blood pressures had previously been normal Eight of the latter had 100 mm Hg diastolic pressure the value of 110 mm Hg was recorded for one single female patient aged 34 years All but two of these nine patients were younger than 40 years and only two of them were males Diastolic hypertension was thus present in 11 subjects preoperatively and in 15 postoperatively The average pulse pressure of all patients was normal although small pulse pressure was a common finding in the patients younger than 40 years

Table 6 The second heart sound on auscultation before and after operation for ASD secundum in 101 subjects younger than 40 years and 28 subjects 40 years of age or older Three cases of residual shunt are included, two of them presenting a small ( ) and one a large (**) shunt

	Age	N	II sound					II P accentuated
			Single	Narrow split		Wide split		
				changing	fixed	changing	fixed	
Before operation	< 40	101	1	3	10	8	79	23
	≧ 40	28	—	2	2	1	23	12
	All	129	1	5	12	9	102	35
After operation	< 40	101	3	52*	2	35*	9**	10
	≧ 40	28	5	14	3	5	1	2
	All	129	8	66	5	40	10	12

Palpation

Fig 4 presents the preoperative and postoperative frequencies of palpable impulses of different origin and different strength in the young and old age groups of patients Data on the preoperative palpation findings made in different types of pulmonary circulatory disorders have been compiled in Table 5 (p 40)

Impulse of the right ventricle — The impulse was normal preoperatively in 5 % only and postoperatively in 77 % On the average it was somewhat more heaving in the group of older patients and it was more intense in cases of more severe pulmonary vascular disease In a small fraction of the cases it was also laterally palpable beyond the midclavicular line The 15 patients with pulmonary vascular disease and/or pulmonary hypertension all had a normal impulse postoperatively except for three whose impulse was of grade 3 Two of the latter still presented marked obstructive pulmonary hypertension while the third had hyperkinetic pulmonary hypertension due to a large residual shunt The right ventricular impulse of two further cases with small residual shunt was normal

The *apical impulse* was preoperatively palpable in only 7 % of the subjects younger than 40 years but in 25 % of those aged 40 years or older The corresponding postoperative figures are 9 % and 15 % respectively Considering the entire series the impulse was felt in about 10 % both preoperatively and postoperatively Only about half of the patients with preoperative elevated diastolic blood pressure had a normal apical pulse while in the rest it was of grade 1/3 This same grade of impulse was noted only in one-quarter of the subjects with elevated diastolic blood pressure at follow-up

The impulse of the pulmonary artery was normal in 66 % preoperatively and in 94 % postoperatively The grading of the impulse in the group of patients with pulmonary vascular disease and/or pulmonary hypertension did not differ on the average from that in the rest of the series

The impulse due to closure of the pulmonary valve was preoperatively palpable in 30 % of the cases it was more intense in cases with incipient pulmonary vascular disease and hyperkinetic or obstructive pulmonary hypertension In all such cases expect for one presenting obstructive pulmonary hypertension the impulse was postoperatively impalpable

Thrill over the pulmonary area was felt in 16 % of the cases more often in subjects younger than 40 years It was never of grade 3/3 grade 2/3 occurred in two patients whose systolic pulmonary gradient was 7 mmHg and 38 mmHg respectively

Auscultation

The *first sound* was characterized as normal or as loud with or without split Aural judgement of the presence of split was often dif-

ficult if one of the components of the first sound was loud Preoperatively the first sound was normal in 62 % in the rest it was loud with or without split split occurring commonly The finding of loud first sound was more prevalent (54 %) in the group of patients aged 40 years or older The first sound was postoperatively normal in 71 % and 27 % presented split without accentuation of any component It was assessed to be loud in only three subjects of whom one had a large residual shunt In the group composed of patients with follow-up times less than two years split first sound was postoperatively more than twice as common as in the complementary group

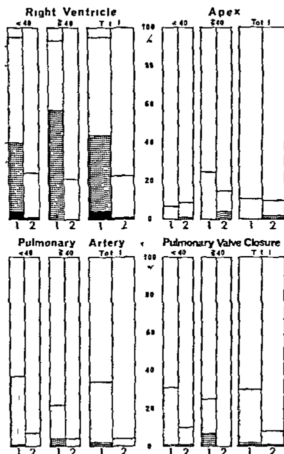


Fig 4 Frequencies in percent of right venticular apical pulmonary artery and pulmonary valve closure impulses felt by palpation before (1) and after (2) operation for ASD of secundum type in 101 subjects of the young age group and 28 of the old age group — Blank Normal impulse Singly hatched Impulse of Grade I Cross hatched Grade II Black Grade III

The *second sound* was classified by the single and narrowly or widely split types the split was either fixed during respiration and Valsalvas manoeuvre or changeable The second (pulmonary) component of the sound (II P) was considered to be accentuated if its loudness was estimated to be at least twice that of the first component Tables 5 and 6 show the findings in regard of the second sound Accentuated II P was thus preoperatively noted in only 35 cases (28 %) more often in the older than in the younger patients (43 % and 23 % respectively) In the presence of pulmonary vascular disease too somewhat higher frequency of accentuated II P was found apparently depending on the severity of the disease Only 9 % of the cases presented loud II P at follow-up Narrow or wide split of the second sound was preoperatively present and fixed according to aural assessment in 114 cases (88 %) if the instances of wide but changing plit are included the cases with abnormal second the instances of wide but changing split are second sound occurred a follow up in 15 cases (12 %) only but counting also the 40 cases in which wide changing split wa observed the percentage of patients with postoperatively abnormal second sound is 43 % One of the three cases with residual shunt displayed fixed wide split while in the other two the split was aurally variable and the second sound was not distinguishable from normal by auscultat on

The third sound was heard in 13 patients (10 %) preoperatively and in four cases postoperatively Its origin was presumably the right ventricle judging from the point of maximum audibility medial to the apex The presence of the sound was not related to the symptoms or signs of heart failure or to any other finding recorded in this study

The *fourth (atrial) sound* was heard medial to the apex in 17 cases (13 %) before and in six after surgery all the cases in which it disappeared belonging to the group of patients younger than 40 years Patients with audible fourth sound were all free of pulmonary vascular disease and their pulmonary-to-systemic flow ratio did not differ at any statistically significant level from that of the entire series (3.37 and 3 55 respectively) The

peak pressure of the *a* wave in the right atrial tracings made of these cases during catheterization was not different on the average from that recorded in the other cases of the series

Early diastolic sound was noted in 2 % of the preoperative and in no less than 9 % of the postoperative auscultations with equal incidence in both age groups In all instances, it was heard as a high-pitched snap parasternally on the left side in the third to fifth intercostal space It is likely that the same sound has been taken for tricuspid inflow murmur in a number of cases

Pulmonary ejection sounds were heard in the group of patients younger than 40 years in 11 % before and in 15 % after surgery The corresponding percentages for the old age group are 18 % and 39 % respectively The three cases with obstructive pulmonary hypertension all presented this sound The prevalence in instances of less severe pulmonary vascular disease was in the same order as in the series as a whole Usually ejection sounds occurred over the pulmonary area but in some instances they were heard as far down as the fourth or fifth intercostal space parasternally on the left

Systolic murmur in the pulmonary area was preoperatively present in the entire series without exception It persisted postoperatively in 43 cases (33 %) of which six belong to the old and 37 to the young age group (21 % and 37 % respectively) The murmur elicited before surgery was characteristically of ejection type in the majority (66 %) of grade 3/6 and rarely of grade 1/6 or 5—6/6 (both in 1 %) After surgery the murmurs were predominantly (in 25 out of 37 cases) of grade 1/6 their presence and intensity had no relation to postoperative presence or absence of pulmonary systolic gradient except in one single case presenting murmur of grade 3/6 in which a systolic pulmonary gradient of 38 mm Hg had been established preoperatively

Apical systolic murmur was heard in 12 cases (9 %) preoperatively and in the same number of cases also after the operation Its loudness was grade 3/6 in two cases only in one of these which had a history of rheumatic fever the operation revealed slight deformity of the mitral valve

Tricuspid systolic murmur was preoperatively displayed by one patient younger than 40 years and by three of the older ones It did not occur postoperatively in any of the latter while four of the younger subjects had such murmur after surgery It was not associated with any abnormalities of the jugular venous pulse contour

Early diastolic murmur of pulmonary regurgitation was only noted in two cases persisting after surgery in one of them in which it was due to valvular deformity probably produced by bacterial endocarditis

Early diastolic murmur of aortic regurgitation was heard and also established by phonocardiography in one instance after surgery

Delayed diastolic apical murmur resembling that of mitral stenosis was preoperatively present in six cases but completely absent postoperatively The mitral valve was found to be normal at operation in all six cases

Delayed diastolic medial murmur considered to be a tricuspid inflow murmur was preoperatively heard in 86 cases (67 %) about twice as commonly in the group of patients younger than 40 years as in the older group (74 % and 39 % respectively) It was mostly of grade 2 (in 49 cases out of 75) or grade 1 (in 30 out of 75) There was only one postoperative occurrence in a patient with residual left-to-right shunt The murmur was usually high pitched sometimes of scratching character and sometimes initiated by a snap In some instances it resembled the rumble in mitral stenosis and was transmitted towards the apex

Comments on the physical findings

The jugular venous pressure was elevated in 14 cases which also displayed other signs of systemic venous congestion Considering the anamnestic data suggesting systemic venous congestion there were altogether 32 patients meeting the respective criteria which is 25 % of the entire series The group of patients younger than 40 years contained 15 such individuals (15 %) and that of older patients 17 (61 %) Postoperatively such symp

toms and signs were only present in one subject of the younger and four of the older group Of the 32 patients 14 suffered from more or less severe congestive heart failure on admission for treatment in the other 18 patients with history of heart failure no signs of it were found or they were inconclusive The group of 14 patients with clinical heart failure includes only four who had arterial hypertension in most cases slight to moderate in degree Three of them and one further patient had anginous chest pain which may have been related to coronary disease However all these patients were females between 40 and 60 years of age and they had no angina after surgery None of them had any mitral or aortic valve disease Definite pulmonary vascular disease was present in six cases two of them also afflicted with angina and systemic arterial hypertension Thus if left ventricular affection is considered to be conditional for congestive heart failure in ASD clinical evaluation disclo ed no plausible explanation for the condition of nine out of the 14 cases with heart failure in the present series and in the rest the relationship seems to be dubious owing to the moderateness of the disorders involved

Palpatory and auscultatory methods are rather satisfactorily able to distinguish between uncomplicated and complicated cases of ASD in view of the fact that for bedside diagnosis additional information from history electrocardiography and radiology is available The right ventricular impulse in particular and the impulse from closure of the pulmonary valve possess discriminating value as do the pulmonary component of the second sound (II P) and the pulmonary ejection sounds All these excepting the ejection sounds are likewise valuable in assessing the result of surgery owing to their considerable tendency of normalization However ejection sounds and palpable pulmonary valve closure are perhaps more positively related to the size of the pulmonary artery than to pulmonary pressure (Leatham and Vogelpoel 1954) This is substantiated by the fact that in the present cases with accentuated II P the size of the pulmonary artery seen in the radiogram was grade 2/2 in 77 % as compared to 45 % in cases with normal II P When there were ejection sounds grade 2/2 of the pulmonary artery size was noted in 75 % against 43 % in the entire series This correlation on the anatomy of the pulmonary artery imposes some limitations on the usefulness of the methods in question and this is in fact the main cause for the considerable overlapping of the present findings which is particularly evident in cases with small residual shunt No discriminating capacity could be attributed to the impulse of the pulmonary artery

The split second sound encountered at auscultation has no particularly good power of discrimination between cases with and without residual shunt seeing that in two cases with small residual shunt the aural impression was one of changing split with respiration while on the other hand fixed narrow or wide split was postoperatively heard in 14 cases which had no shunt This inconsistency is probably partly accountable to the tendency of auditory observation to exaggerate small changes in time intervals in addition to which its power of resolution of such changes is subject to external interfering effects particularly to noise The value as a discriminating indication of the second sound shall be further discussed in connection with the phonocardiographic findings in which association also the behaviour of the first sound will be considered

Pulmonary systolic murmur was postoperatively heard in 33 % only Petersson (1967) found systolic murmur after operation in 90 % of his cases half of which were children Loogen et al (1961) report postoperative systolic murmur in about half of their series of which one-quarter were children Phonocardiography confirmed the absence of pulmonary systolic murmur in 77 % of the present cases No obvious explanation suggests itself for this discrepancy of results but the difference in age distribution may be partly responsible The fact that in the present series murmur was nearly twice as frequent among those younger than 40 years as in the older group points in this direction

In two out of four cases with postoperative tricuspidal systolic murmur no explanation other than the anatomical changes in the atria can be tendered for the symptom By analysis of atrial pressure graphs and by thermodilution studies Loogen et al (1961) showed that slight tricuspidal incompetence is not uncommon after surgical correction of ASD Similar valvular dysfunction presumably representing a stretch effect was also noted postoperatively in one case of the present series in the aortic valve

GENERAL COMMENTS ON SYMPTOMS AND PHYSICAL SIGNS

When clinical cardiology was still in its early stage the sole important clue for diagnosis of ASD secundum was considered to be anamnestic history of fair functional capacity retained up to advanced age regardless of large heart, of presence of murmurs and of dyspnoea on exertion. The physical findings were not thought to contribute much to the diagnosis (e.g. Burrett and White 1945). By now sufficient experience has been accumulated to enable correct diagnosis to be achieved on the basis of the bedside findings in most cases of ASD. The spectrum of clinical profiles encountered in ASD at different ages and in the presence or absence of complicating conditions is varied. Its main characteristic is slow impairment of symptoms and signs. The rather typical auscultatory and palpatory findings are fairly constantly encountered at all stages of the disease and they are of decisive value for diagnosis, particularly in combination with the history and radiogram, which were mentioned above, and with the electrocardiogram.

The result noted after technically successful surgery was almost invariably favourable in the present series of 28 patients aged 40 years or older and of 101 patients between the ages of 15 and 39 years regardless of the length and nature of their previous medical history. Impairment only ensued in one single case in which apparent coronary disease was present. It is difficult to assess the results quantitatively since such information as has been available is often coloured by the patient's outlook. But also unbiased rating has been possible to some extent. Thus the level of functional capacity after surgery seems to be partly dependent on the preoperative level. In all those cases in which pulmonary vascular disease was present the pertinent symptoms and signs were attenuated, in cases of heart failure the clinical signs disappeared, and the classical symptoms of left or right heart failure disappeared in the majority of the cases. A good clinical impression is thus gained of the results of surgery regardless of the patients' age.

SYNOPSIS

The pertinent symptoms recorded in 129 cases of ASD of secundum type were analyzed, in particular in terms of the age distribution of their appearance. Dysrhythmias increased with increasing age until atrial fibrillation made its appearance, and both are associated with subjective and objective deterioration of the patient's condition. One-fifth of the patients deteriorated as far as Functional Class 3 (FC3) (N.Y.H.A.) which they entered at an age between 30 and 50 years. Patients aged 40 years or older accounted for 52 % of this class and for 100 % (4 cases) of those in FC4. The appearance of most symptoms in relation to age occurred bimodally in the present material, constituting a sign of its heterogeneity: the first mode was usually seen in the first decade and the second in the third decade or later. The frequency of onset of symptoms related to heart failure too yielded a bimodal age histogram; both modes were late and neither one was markedly related to development of pulmonary vascular disease.

The prevalence of symptoms at follow-up was low, as a rule less than 15 %, and the proportion of patients preoperatively belonging to FC 2, 3 or 4 who improved by one class or more was 84 % and 94 % of the old (40 years or more) and young age group, respectively. Only two patients showed impairment owing to a complication of operation and to coronary heart disease, respectively.

The information elicited by palpation and auscultation seemed to possess some value as regards differentiation between uncomplicated and complicated ASD and also in distinguishing the cases presenting a successful result of surgery and those with residual shunt. On the whole the result of surgical correction of ASD of secundum type was considered, on clinical grounds, to be good in patients aged 40 years or older although this group displayed a tendency of somewhat higher residual symptom frequency than the complementary group of younger patients.

VI PHONOCARDIOGRAPHY

METHODS

Equipment

Phonocardiographic recordings were made of the heart sounds of the patients in the present series, using either a piezoelectric contact or an air chamber microphone The postoperative graphs were obtained exclusively with the aid of a Type EMT 25 pickup by Elema Schonander (Sweden) wich is known for its good high frequency response Logarithmic amplifiers were used provided with high pass filters having nominal frequencies of 35 70 140 and 250 Hz according to Maass and Weber and in most instances also with filters having 400 and 140 Hz nominal frequency the latter similar in its other characteristics to the 0 isophone of Fletcher and Munson Some of the preoperative recordings were made using octave filters with the nominal frequencies 12, 25 50 100 200 and 400 Hz and a 0-isophone filter Three different types of recorder were preoperatively applied a direct writing heated stylus recorder (Cardiopan 3 and 6 Philips, Holland/Switzerland) with linear response up to about 150 Hz, a direct writing ink jet apparatus (Mingograf 24B Elema Schonander Sweden) with linear response up to 500 Hz, and a high frequency photographic film recorder (Klinik Elema Schonander Sweden Atlas 4 Atlas Werke Germany) The postoperative phonocardiograms were all produced with a Mingograf 81 six channel ink jet recorder (Elema Schönander Sweden) presenting linear response up to 500 Hz and its amplifier provided with six high pass filters producing a spectrum of bands as suggested by Maass and Weber and as specified above All types of equipment had a time constant in the order of 2 seconds

Procedure

All phonocardiographs were taken in supine position during quiet breathing, over a couple of deep respiratory excursions, and also immediately after Valsalva s manoeuvre Phonocardiograms were taken in this manner from the aortic pulmonary tricuspid and apical areas when there were sounds or murmurs having their point of maximum intensity somewhere else recordings were also made from such points Several respiratory cycles were covered by each record The paper speed was 100 mm/sec with rare exceptions only The evaluation included examination and measurement of 10—20 cardiac cycles in order to exclude potential artefacts and spurious variations of the variables involved

Comments on phonocardiographic methods

It is well known that in respect of high frequency sounds and murmurs even good phonocardiographs cannot surpass the performance of the human ear (Leatham 1952, Luisada and DiBartolo 1961) which operates at highest acuity in the neighbourhood of 2000 Hz and, so far excels over any kind of electric equipment in capacity and usability for selective detection and interpretation of sounds and murmurs. In contrast, sounds of 50 Hz or lower frequency are poorly audible and those below 30 Hz are mostly not perceived at all. Thus in the frequency range under 100 Hz phonocardiography is far superior to auscultatory detection of sounds As a rule most heart sounds yield the best recording in frequency bands below 100 Hz, but occasionally also sounds of higher frequency (various kinds of snaps and clicks) are noted Systolic murmurs mostly appear on bands lying between 200 and 400 Hz, but early diastolic, high pitched murmurs often have a frequency in excess of 400 Hz.

Different thoracic tissues have different damping characteristics and the intervening tissue surfaces differ in their reflecting properties, with the result that the heart sounds and murmurs are transmitted in different manner to the extrathoracally applied microphone depending on the amount and kind of tissue interposed between the sound source and the monitored skin area, as Feruglio (1962) has shown. This implies that changes in heart size location of the heart in the thorax and postoperative pericardial, mediastinal, pleural and, potentially lung parenchyma changes are factors seriously limiting the quantitative comparability of preoperative and postoperative phonocardiographic findings. For this reason the time relationships of the sounds elicited by phonocardiography were the sole aspect subjected to analysis in this study while less heed was paid to the acoustic amplitudes of the murmurs and heart sounds

FINDINGS

Phonocardiographic records were made preoperatively in 15 cases and postoperatively in all 129 cases of the present series

The *murmurs* have been considered in the preceding chapter dealing with the auscultatory findings No particular analysis of their occur-

rence or character was undertaken on the basis of the phonocardiograms On the whole good agreement between the auscultatory and phonocardiographic observations relating to murmurs could be noted in that in all instances in which a murmur was revealed by the record it was also independently heard and *vice versa* *Heart sounds* however were revealed by phonocardiography with slightly higher sensitivity than by auscultation

First heart sound

The first sound displayed an abnormally loud first and particularly second component (I a and I b) in all preoperative records in four of them the third component (I c) was accentuated and high in frequency with 160—175 msec Q-I c interval

Postoperatively splitting of the first sound was heard and/or recorded in altogether 52 cases (in 42 % of all cases but those with residual shunt) The postoperative findings have been compiled in Table 7 In the great majority of cases with auscultatory impression of split first sound it was not accompanied by any marked broadening of the I a—I b interval but occurred together with an ejection sound clearly visible in the phonocardiogram in 42 % In 18 out of 52 cases I c consisted in the phonocardiogram of a prolonged series of low frequency vibrations from 110 to 140 msec after onset of the Q deflection in the electro cardiogram and exact timing of the Q—I c interval was not possible I c was both heard and recorded in 13 cases it was not heard but only phonocardiographically recorded in 21 cases Thus in 35 % of altogether 52 cases split was heard but not adequately recorded in 25 % it was heard and recorded as late I c and it was not heard but recorded as late I c in 40 % In the last of these three groups I c was mostly recorded on the low-frequency bands while audible Ic usually also appeared in the medium-frequency bands The other two components I a and I b appeared at an average of 60 msec and 90 msec respectively after onset of Q

Table 7 Phonocardiographic and auscultatory findings in 52 cases with split first sound after surgical closure of ASD secundum with special reference to timing of the ejection component (Ic) and effect of the length of the follow-up period and of age

	Age < 40 years $N = 98$			Age ≧ 40 years $N = 28$		
	N	% of findings	Mean Q—Ic (msec)	N	% of findings	Mean Q—Ic (msec)
Ic heard and recorded timing indeterminable	16	50 %		2	10 %	
Ic recorded and timing determinable	16	50 %	167 ± 23 6	18	90 %	156 ± 18 2
Heard	3	9 %		10	50 %	
Not heard	13	41 %		8	40 %	
Total (% of age group)	32 (31 %)	100 %		20 (71 %)	100 %	
Follow-up period less than 30 months						
Recorded and or heard	17	53 %		11	55 %	
Recorded only	5	16 %	175 ± 7 7	10	50 %	150 ± 15 4
Follow-up period 30 months or longer						
Recorded and or heard	15	47 %		9	45 %	
Recorded only	11	34 %	163 ± 27 3	8	40 %	163 ± 18 7

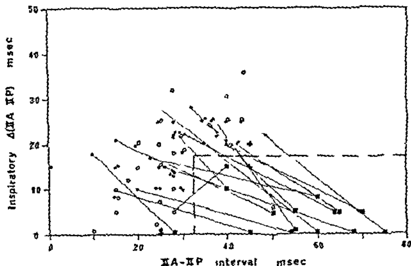


Fig 5 Splitting of the second heart sound in ASD of secundum type Time intervals IIA—IIP measured at the end of deep expirium plotted against their corresponding increase at the end of deep inspirium or immediately after Valsalva's manoeuvre — Square Before operation Circle After operation (Open — old age group Filled — young age group) Cross Residual shunt

Second heart sound

The second sound was widely split and fixed in all but one of the preoperative records in which it was fixed but only 24 msec in width The case in question was one with congenital atrioventricular block and nearly normal pulmonary pressure The other cases all presented a split in excess of 30 msec which was augmented by less than 20 msec on inspiration

Postoperatively the split was usually less than 30 msec and its inspiratory increase was usually 20 msec or more Whenever the split was more than 30 msec its inspiratory increment exceeded 20 msec in all but one case the patient in question being one who had complete right bundle block at operation after merely slightly prolonged QRS preoperatively In the majority of the cases the inspiratory increase of split amounted to more than 40 % The results have been shown in Fig 5 in which the II A—II P interval at the end of the expirium has been plotted against its inspiratory increment in msec Of the three cases with residual shunt two had a split amounting to 45 msec with inspiratory broadening by 15 msec and 20 msec the latter being a case in which the residual shunt was small with less than 20 % of pulmonary flow The split was fixed in the third case but only 25 msec in amount This patient had a large residual shunt and pulmonary hypertension

COMMENTS ON PHONOCARDIOGRAPHIC FINDINGS

The first heart sound is composed of three components usually denoted with I a I b and I c Earlier the first two were thought to be related with the closure of the mitral and tricuspid valves whence the symbols M and T are still widely used This concept has been invalidated by recent investigations and there is evidence that in the normal heart and also in some pathological conditions I a and I b are related with the isovolumic contraction of the left ventricle the amplitude of I b being positively correlated with the amplitude of systolic ascent of the first derivative of left ventricular pressure (Shah et al 1963 van Bogaert 1966) The contribution of the right ventricle is minimal in normal cases (van Bogaert 1966 Luisada et al 1967) and even in those with mitral stenosis (Kurz et al 1967) Right bundle branch block does not affect the components of the first sound but in left bundle branch block all three are delayed (Oravetz et al 1967) and the same is true in other conditions causing delay in the initial part of the left ventricular contraction (Kurz et al 1967)

In atrial septal defect the I a—I b interval is usually slightly prolonged its increment being proportional to the magnitude of the left to-right shunt likewise the amplitude of I b is found to be proportional to pulmonary

flow in ASD (Kubis et al 1967) Reversal of the normal amplitude pattern of I a>I b is suggested to be a useful sign in the diagnosis of ASD (Lopez et al 1962) All these phenomena have been attributed to the increased volume load causing prolonged ejection of the right ventricle They may be ultimately accountable to unusually large contribution of the right ventricular hypertrophied muscle mass to generation of the first sound It might be possible on the other hand to explain the first sound pattern in terms of left ventricular function since the lowered end-diastolic volume of the left ventricle in open chest dogs may delay the high frequency (I b) vibrations and sometimes increases the amplitude of the sound (van Bogaert 1966) and since the diastolic filling of the left ventricle has mostly been stated to be low in ASD

The concept that the early systolic sounds are related to dilatation of the pulmonary artery and to pulmonary hypertension has been generally accepted ever since the analysis of these sounds by Leatham and Vogelpoel (1954) It has been shown in the laboratories of Luisada and van Bogaert that this sound is a constant element of the first sound occurring normally 125 ± 3 4 msec after the onset of Q (Oravetz et al 1967) and it is attributed to turbulence of blood and wall vibrations in the left ventricular outflow It is not affected by right bundle branch block whereas in left bundle branch block it is delayed to about 170 msec after onset of Q According to the authors cited splitting of the first sound is not related to potential presence of bundle branch block but rather to an accentuated I c component and this is consistent with the present observations It has been stated that in ASD the ejection sound is easily confused with a split first sound because it is often also audible in the tricuspid area (Schrire et al 1963) or also because the split is audible in the pulmonary area (Kelly and Lyons 1958) or differs little from normal on the whole (Eisenberg and Hultgren 1959) It seems that the disagreement rather centres on what phonocardiographic detail should be specified as the equivalent of a split sound heard at auscultation It is likely that prolonged I a—I b time and prolonged I b—I c time may both produce the auditory impression of split depending e g on the location of the stethoscope or recorder head, on the characteristics of transmission etc In the present series, the split presented best correlation on presence of an accentuated and delayed ejection component in the first sound Since there is no reason to suspect that the left ventricular contraction had been postoperatively delayed the origin of this component was in all likelihood right ventricular outflow in the present cases Some support is lent to this assumption by the finding that out of 34 cases with delayed I c in the phonocardiographic record nine had a preoperative pulmonary vascular resistance index in excess of 250 dynes sec/cm⁴, while it was less than 100 in two cases only The pulmonary artery was also larger in the cases with loud I c and with distinct auscultatory split

The length of the follow-up period also has some bearing on the occurrence of split first sound The sound was more commonly encountered in cases with a follow-up period less than 30 months than in those whose follow-up period was 30 months or longer Split was present during the first postoperative year in 7 out of 15 cases but in only one out of 38 who were followed for more than three years Cases with ejection components inaccurately distinguishable in the phonocardiogram in spite of their audibility, decreased in proportion with increasing length of the follow-up period They may be considered in great likelihood to be composed of both left and right heart ejection components whereby they would constitute a transitional form towards the normal finding Despite its probable relation to the involution process of the right ventricle the postoperative trend towards normal of the split first sound bore no relation to the postoperative changes in amplitude of R or of R in V_1 There was however some relation to the duration of QRS in that the prevalence of right bundle branch block of Grade 3 was in the group with longer follow-up periods about one-third of that in the group with follow-up periods less than 30 months

The earliest observations on split second heart sound are those reported by Nerard (1948) and by Barber et al (1950) This particular abnormality of the second sound has ever since been rated as one of the most characteristic auscultatory features in ASD It has been attributed to lack of the normally opposed effects of respiration on the filling of the right and left ventricles resulting from the physio logically common atrium (Aygen and Braun

wald 1962) In the present series the width of the split had no relation to the magnitude of the shunt in conformity with findings made by Reindell et al (1962) and by Castle (1967) but it was often smaller in cases with elevated pulmonary pressure as previously Jonsson (1958) and Castle (1967) have noted As a rule widely split second sound is encountered in about 95 % of cases with ASD and its postoperative presence has generally been considered a sign of probable residual shunt The respiratory changes of the split have been examined during deep inspirium as well as immediately after Valsalvas manoeuvre without eliciting any noteworthy difference (Aygen and Braunwald 1962 van der Hauvaert 1964 Wennevold 1967) identical results were obtained by both methods in the present work The split became postoperatively more movable during respiration and narrower on the average It possesses considerable value as a discriminant of residual shunt cases but there is overlapping Thus in a case with residual shunt and pulmonary hypertension a split was observed which was fixed but normal in width while in another case with minor residual shunt the split displayed an inspiratory increase of 20 msec Moreover one case free of residual shunt but presenting complete right bundle branch block as a complication of the operation had a postoperative fixed split of 50 msec A similar case was recently reported by Luisada et al (1966) The width of the split was related to the QRS duration to some extent as also Loogen et al (1961) have stated However no mathematical relationship was found which is consistent with the observations of Petersson (1967)

SYNOPSIS

The preoperative phonocardiographic records of 15 cases of ASD and those made after surgery in 129 cases were analyzed in respect of the behaviour displayed by the first and second heart sounds

The first sound commonly presented split on auscultation both preoperatively and postoperatively after surgery more frequently in the group of patients aged 40 years or older Occurrence of auscultatory split was best related to the delayed and often accentuated ejection component of the first sound The frequency of split decreased with time elapsed after surgery this may be one of the signs indicating right ventricular involution although there is no correlation with electrocardiographic involution

The second heart sound showed more than 30 msec split preoperatively its inspiratory increase was less than 20 msec Postoperatively, the split of the second sound was less than 30 msec and/or its inspiratory increment equalled or exceeded 20 msec The increase at the end of a deep inspirium surpassed 40 % in the majority of cases However there was overlapping as regards discrimination of residual shunt cases on the basis of the behaviour displayed by the second sound False positive indication was obtained in a case with complete right bundle branch block and false negative indication in a case with residual shunt and pulmonary hypertension as well as one further case presenting a residual shunt with less than 20 % of pulmonary flow

VII ELECTROCARDIOGRAPHY

METHODS

Equipment

Substantially most of the preoperative electrocardiograms and all postoperative ones were recorded by means of a direct writing ink jet device (various types of the Mingograf Elema Schonander Sweden) while in comparatively few instances a set up with photographic film recorder (Atlas 4 Atlas Werke Germany Klinik, Elema Schonander Sweden) was employed Furthermore a direct writing heated stylus device (Cardiopan 3 or 6 Philips Holland/Switzerland) was used preoperatively in about half of the cases for repeated routine electrocardiography in the ward All types of apparatus had a time constant in the order of 2 msec most likely this implies 3 db low frequency cutoff at a frequency lower than 0 1 Hz, assuming 6 db rolloff per octave (as calculated according to Berson and Pipberger 1966 a) The high frequency 3 db cutoff of the heated stylus recorder was 160 Hz according to the manufacturer s statement, and its linear recording width was 70 mm The ink jet electrocardiographs had their 3 db high frequency cutoff at 650 Hz and virtually linear response up to 500 Hz, their linear recording (with 35 mm jet length) covering a 55 mm range

Procedure

The recordings were invariably performed as hospital routine examinations in the ward, except in the case of the special atrial electrocardiograms taken postoperatively in connection with phonocardiography by the author The gain used in these latter instances was one producing 35 mm deflection with 1 mV calibration potential and the paper speed was 100 mm/sec the corresponding data in all other recording runs were as a rule 10 mm deflection for 1 mV and 50 mm sec paper speed The conventional twelve lead system was applied in all cases standard bipolar and augmented unipolar limb leads and six unipolar chest leads (I II III aV_R aV_L aV_F V_{1-6}) For atrial electrocardiograms the standard limb leads and the V_1 unipolar chest lead were used The recording was always made with the patient in supine position Plate electrodes rubbed with electrode jelly were used throughout For check on the synchronism of the channels and of appropriate gain, a calibration potential of 1 mV was applied at the end of each strip

Measurements

Several cycles were measured Whenever asynchronism or inappropriate gain was noted, the resulting error was taken into account in the time interval and potential measurements. In respect of time intervals an accuracy of 5 or 10 msec could be achieved when the paper speed was 100 or 50 mm/sec respectively The accuracy in amplitude achievable with the aid of a magnifying glass was 0 03 or 0 01 mV with a gain equivalent to 10 mm and 35 mm per 1 mV respectively For base line in measuring the amplitudes of deflections the P R segment was used

Mean frontal axis — The net area between the curve and the base line was estimated in mm² using a transparent squared plate The net areas of the deflections were estimated in the two standard leads yielding the largest deflection, and the orientation of the mean frontal vector was determined in usual manner by plotting the values of deflection area on Einthoven s triangle (e g Lamb 1965) Both AP and AQRS were thus determined preoperatively and postoperatively in all cases Furthermore the orientations of the mean frontal vectors of the right and left atrium were separately determined according to Gooch et al. (1966) with some modifications AP_{RA} was obtained by measuring the height of the P wave at the point 30 msec after its onset in the synchronous P deflections on two standard leads or two unipolar limb leads and by the usual procedure of axis determination from these values with the aid of Einthoven s triangle AP_{LA} was found in equivalent manner employing the height of the P deflection measured at the point 30 msec short of its end In the statistical analysis of the changes of axis the vectors were treated as equipotential vectors and from the circular distribution of their end points the resultant vector was calculated the magnitude of this resultant was further evaluated to furnish an approximate assessment of the angular dispersion (standard deviation) (Siltanen et al in preparation see also Appendix p 151) The error inherent in the method was about ± 10 degrees in the atrial axis and about ± 5 degrees in the QRS axis determinations For statistical analysis the angular values were grouped with 5 degree class intervals

Initial and terminal P forces — The P wave in the V_1 lead was divided into two components for further analysis at the point of minimum amplitude in cases with bimodal P and at the crossing point with the base line in instances with biphasic P

Both subdivisions of the wave were evaluated in terms of amplitude times duration (mm × sec). These integrals, known as the initial and terminal P V_1 forces or indices (Morris et al. 1964) were calculated from the routine electrocardiograms preoperatively and postoperatively in all cases, furthermore they were derived from all postoperative special electrocardiograms made with higher gain and greater paper speed. When there was atrial fibrillation the amplitudes of the fibrillatory waves were measured (Peters et al. 1966).

Other measurements — The following measurements were made in addition to the axis determinations and determinations of P forces: amplitudes of P(II), R(V_1), S(V_1) and S(V_5), P-R interval, duration of P(II), upstroke time of P(II) from its onset to its maximum amplitude, duration of QRS(I-III), QRS(V_1), QR(V_1) and S(V_5). The QR/QRS(V_1) and R/S(V_1) or R'/S(V_1) ratios were calculated. The extent of primary T changes in the chest leads, if any, and the site of the transition zone recognized in the trabecular or biphasic equipotential pattern (Lipman and Massie 1965) were analyzed.

P and QRS morphology — The preoperative and postoperative electrocardiograms were analyzed for the morphology of the waves. P(V_1) was classified by nine types according to all possible sequence combinations of the +, 0 and — pattern in the two consecutive subdivisions of the wave. QRS(V_1) was classified according to the height of each deflection. Any deflection 0.5 mV or higher in amplitude was given the score of 2 and it was denoted with a capital symbol because this value represents the approximate upper limit of the normal r wave in V_1 (Simonson 1961, Burch and DePasquale 1967), i.e. Q, R, S, R' or S', respectively. The score of 1 was assigned to deflections less than 0.5 mV in amplitude and lower case symbols were used for them: q, r, s, r' and s', respectively. Whenever a given deflection was absent it received the score of 0.

Frontal QRS sÊ loop — The frontal loops were manually plotted according to the method of Grant and Estes (1951) but using another planar frame. Electrocardiograms obtained from two synchronous channels were thus treated, mostly only two of the standard leads were employed but occasionally other combinations of the standard or unipolar limb leads were needed. The voltage values were read at synchronous points in each lead with 10 msec intervals and plotted on a corrected hexaxial frontal plane frame which had been derived from Burger's average scalene triangle as calculated by Massie and Walsh (1960) from the results of Frank and Kay. The perpendicular on the axis was drawn at either one of each pair of points, and the intersections of perpendiculars in consecutive voltage pairs were connected to form the loop.

Interpretation

Conduction — P-R intervals not exceeding 220 msec were considered to be normal. The right intraventricular delay was classified by three degrees (Lipman and Massie 1965): Grade 1 implying terminal notching of the ascending limb of S(V) and some broadening of S($V_{5,6}$); Grade 2 an RSR' pattern in V_1 with QRS less than 120 msec in duration and with broadening of S($V_{5,6}$) (i.e. the pattern of "incomplete right bundle branch block"); and Grade 3 an RSR' pattern with 120 msec QRS duration or longer and with broad S($V_{5,6}$) (i.e. the pattern of "complete right bundle branch block"). 100 msec QRS duration or less was regarded as normal in adults (Simonson 1961, Burch and de Pasquale 1967).

Atrial electrocardiogram — P(II) duration is normally less than 120 msec in adults (Graybiel et al. 1944): 95 msec (S.D. 16) (Pyörälä et al. unpubl. observ.), 83 msec (S.D. 16) (Sanchez Cascos and Deuchar 1963), 96—100 msec (S.D. 14—16) (Burch and dePasquale 1967); accordingly, 120 msec or less.

P(II) amplitude: In adults the following averages have been stated: 0.11 mV (S.D. 0.06) (Pyörälä et al. unpubl. observ.), 0.12 mV (S.D. 0.04) (Sanchez-Cascos and Deuchar 1963, Burch and dePasquale 1967); accordingly, it is 0.2 mV or less.

P(II) upstroke is normally 48 msec or less (Sanchez Cascos and Deuchar 1963, Burch and dePasquale 1967).

Â P in the frontal plane lies between —30 and +75 degrees in 95 % and between +45 and +65 degrees in 90 % of normal adults, being usually about +60 degreees (Burch and dePasquale 1967). Sano et al. (1957) give the normal range as from +30 to +100 degrees. The maximal instantaneous P vector lies between +20 and +60 degrees, on the average at +55 degrees (Massie and Walsh 1960). The range from +30 to +75 degrees would thus seem appropriate to express the normal variation.

Â P_{RA} (the right atrial component of Â P) is normally not different, as a rule, from either Â P or Â P_{LA} according to Gooch et al. (1966), who never found any Â P_{LA} under 0 degrees. Haywood et al. (1966) analyzed the atrial vectorcardiograms of 100 normal persons. Calculating from their data the mass centroids of the frontal plane distributions for the right atrial and left atrial vector components, these are found to differ by about 30 degrees in orientation; the respective absolute values for the right and left atrial vectors are about 70 degrees (range —10 to +100 degrees) and about 40 degrees (range —20 to +80 degrees). Taking into account approximately 80 % of the range, the arbitrary limits of 0 to +75 degrees and 0 to +90 degrees have been set up as reference values for Â P_{LA} and Â P_{RA}, respectively, in the present study.

The P(V) initial force is normally between —0.01 and +0.07 mmsec and the terminal force is between —0.03 and +0.01 mmsec (Morris et al. 1964), or higher than —4 μVsec (Burch and dePasquale 1967), that is, in excess of —0.04 mmsec.

Ventricular electrocardiogram — The QRS(I—III) duration has been considered normal in adults when equal to 100 msec or less (Simonson 1961, Burch and dePasquale 1967) and the QR/QRS(V_1) ratio when in excess of 0.6 (dePasquale and Burch 1963). Â QRS in the frontal plane is between +30 and +85 degrees in 95 % of normal persons, averaging about +65 degrees (Simonson 1961, Burch and dePasquale 1967).

Atrial overloading of various types has no generally accepted criteria (Burch and dePasquale 1967) although criteria associated with the amplitude and upstroke time of P(II) have been suggested for differentiation between pressure and volume overloading of the atria (e g Sánchez Cascos and Deuchar 1963 Anselmi et al. 1968) The duration of the P wave is not affected by right atrial hypertrophy but it is prolonged in left atrial hypertrophy (Macruz et al 1958) though not invariably The P duration correlates positively on the volumes of the atria in particular that of the left atrium but not on their weights, the P amplitude correlates negatively on the atrial volumes but, likewise not on the weights (Gordon et al. 1965) The evidence in favour of relations between atrial hypertrophy and changes in P is thus rather inconclusive In the study of Gooch et al (1965) a leftward shift from +30 degrees of the left atrial frontal plane vector component was the sole sign of left atrial overload in eight out of 28 cases Right atrial overload has usually very little effect on the frontal P vector orientation (Burch and dePasquale 1967) Morris et al (1964) state that more than 90 % of cases with left atrial overload present abnormal negativity in the $P(V_1)$ terminal force this finding has recently been confirmed in children by Reynolds (1967) — The criterion in respect of atrial overloading has been applied partly arbitrarily in the present study that more than one item of those listed in the following is neccessary for any given type of overload to be diagnosed

Right atrial overload (RAO) P(II) amplitude abnormally high P(II) upstroke time prolonged Â P in the frontal plane to the right of +75 degrees Â P_{RA} to the right of +90 degrees Initial $P(V_1)$ force in excess of +0 07 mmsec

Left atrial overload (LAO) P(II) bifid P(II) prolonged past 120 msec Â P in the frontal plane to the left of +30 degrees Â P_{LA} in the frontal plane to the left of 0 degrees. Terminal $P(V_1)$ force more strongly negative than —0 03 mmsec

Right ventricular diastolic overloading was diagnosed in the cases showing RSR pattern in lead V_1 (Cabrera and Monroy 1952 Blount et al. 1957 Burch and dePasquale 1967) provided that there was no complete right bundle branch block and that the QR /QRS(V) ratio did not exceed 0 6 (dePasquale and Burch 1963)

For *right ventricular systolic overloading* a number of electrocardiographic criteria have been presented most of them based on the criteria suggested by Wilson et al (1947) Barker and Valencia (1949) Sokolow and Lyon (1949) and Milnor (1957) The autopsy controlled criteria of Scott (1960 1967) were applied in the present study qR or QR in V_1 R/S or $R/S(V_1)$ in excess of 1 0 provided that the QRS duration is less than 120 msec and the R or R amplitude is 0 5 mV or higher $R(V_1)$ higher than 1 0 mV provided that the QRS duration is less than 120 msec or $R(V_1)$ higher than 1.5 mV when QRS is 120 msec in duration or longer intrinsicoid deflection in V_1 35—50 msec One item was required for diagnosis

Left ventricular overloading was exclusively diagnosed in this study on the basis of chest lead criteria (e g Scott et al 1955) $R(V_{5,6})$ over 2 6 mV $R(V_{5,6}) + S(V_1)$ over 3.5 mV provided that there was no complete bundle branch block

Comments on methods

The equipment used in this study may be considered to possess adequate high frequency response which is important since for instance a fictitious increase or decrease of the R/S ratio by more than 10 % is incurred in 11—16 % of the records if the 3 db high frequency cutoff is as low as 100 Hz (Berson and Pipberger 1967 b) The low frequency characteristics of the equipment were likewise appropriate particularly owing to the fact that the errors caused by low frequency cutoff are markedly less after small or diphasic than after large monophasic deflections the durations of the P waves were probably not affected The high fidelity feature of the ink jet recorder enabled the atrial waves to be examined in closer detail The observations thus made shall not be considered here but it may be mentioned that double determinations from routine and special tracings elicited no significant differences in P area The special electrocardiograms proved to be particularly useful in studying the wave morphology as Brody et al (1967) have pointed out.

The methods employed in measurement and interpretation have been presented and discussed in the foregoing in connection with each item. For a small subseries postoperative frontal qRS s£ loops were plotted both on the standard hexaxial frame and on the corrected frame and compared to the same patients vectorcardiograms (according to Frank s system) It was found that only the loops plotted on the corrected hexaxial frame closely approximated the vectorcardiogram loops.

RHYTHM

Impulse generation

Sinus rhythm was preoperatively present in 99 of the 101 patients younger than 40 years and in 17 of the 28 older ones Atrial fibrillation occurred in one of those without sinus rhythm in the younger group (a male aged 35 years) and in ten of those aged 40 years or older One male patient of 45 had sustained atrial tachycardia with 2 1 atrioventricular block Furthermore one 42-years-old male patient had frequent paroxysms of similar atrial arrhythmia several days in duration each and a woman of 55 years had similar paroxysms of atrial fibrillation One of the younger patients had atrioventricular block of varying degree with temporary episodes of nodal pacing

Postoperatively sinus rhythm was present in 98 out of the 101 younger patients and in

Table 8 Prevalence of right intraventricular conduction delay ("right bundle branch block") of various grades in 126 cases of ASD secundum before and after operation Cases with residual shunt are excluded The prevalences are expressed as percentages of the age group

Grade	Preoperatively %				Postoperatively %			
	0	1	2	3	0	1	2	3
Age < 40	—	1	82	17	1	4	91	4
Age ≧ 40	—	7	61	32	4	4	75	17

23 of the 28 older patients (82 %) Regular rhythm but heterotopic automatism was noted in two patients of these one had a nodal pacemaker and the other one (26 years male 6 years after surgery) displayed at follow-up *left atrial rhythm according to the criteria of* Mirowski (1966) or possibly a pacemaker close to the coronary sinus according to Massumi and Tawakkol (1967) The young patient with atrioventricular block was unchanged postoperatively In one of the older patients with preoperative atrial tachycardia this arrhythmia persisted Of two patients in the older group with debilitating bouts of atrial arrhythmia, one (male) was nearly free of symptoms the other (female) was unchanged Atrial fibrillation was still noted in four patients of whom one (a female patient aged 59 years) had had normal sinus rhythm preoperatively All the persistent arrhythmias were refractory to treatment

Conduction

Sinoatrial block with nodal escape beats was preoperatively found intermittent in one patient It was absent at follow-up like any occurrences noted in other patients during operation or immediately after it

Atrioventricular conduction was preoperatively prolonged in nine out of 100 patients younger than 40 years and in one of 17 older patients with sinus rhythm (9 % and 6 % respectively) Complete atrioventricular block was found in a girl aged 15 years There were no signs of atrioventricular conduction defect in the cases presenting atrial fibrillation Postoperatively the P R time was still found to be prolonged in three cases and prolongation had appeared in another four The conduction defect still persisted in the patient with complete atrioventricular block but it changed to 2 1 block during the exercise test In another young female patient who complained of a *certain degree of residual functional limitation* 2 1 atrioventricular block developed during the exercise test with the bicycle ergometer when the heart rate had reached 142 it subsided when the atrial rate had gone down below 140 The same patient had normal P-R time (190 msec) at rest The P-R times averaged 177 7 msec (range 120—280) preoperatively and 170 7 msec (range 110—250 msec) postoperatively

Pre-excitation was found in two instances one of type B and another of type A (according to Öhnell 1944) which had disappeared by the follow-up examinations

Right intraventricular conduction delay was present in nearly every instance both preoperatively and postoperatively but it abated in 24 cases (Table 8) In one case it worsened from Grade 1 to Grade 2 and in another from Grade 2 to Grade 3 as a complication of surgery There are only two cases in which it became normal

The preoperative QRS duration was 102 6 msec (S.D 15 6) and 107 5 msec (S.D 19 4) in the group of younger and older patients respectively The corresponding postoperative means were 93 7 msec (S.D 11 9) and 96 9 msec (S.D 17 4) No statistically significant differences were established between the age groups The postoperative shortening of QRS duration was significant in the younger group (***) and in the older age group (*) The QRS duration remained as it was in 46 cases and showed some postoperative increase in six.

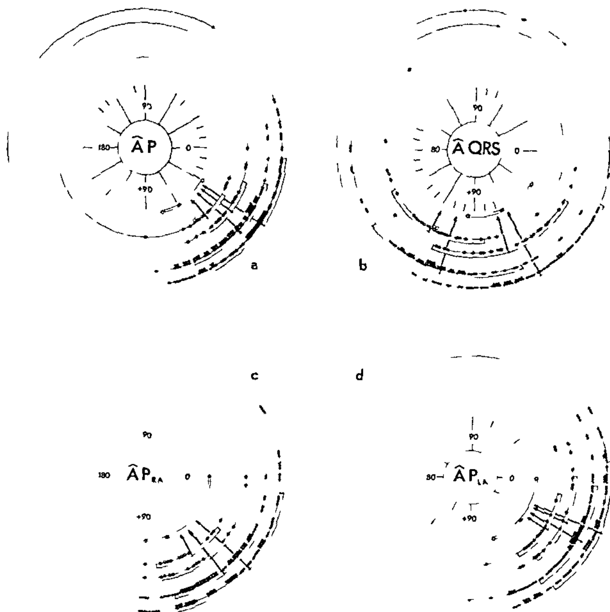


Fig 6 Distributions of mean atrial vectors (Â P Fig 6 a) right and left atrial vector components ($\hat{A}P_{RA}$ Fig 6 c and $\hat{A}P_{LA}$ Fig 6 d) and mean ventricular vectors (ÂQRS Fig 6 b) in the frontal plane scale in 98 patients of the young age group (three cases with residual shunt after operation excluded) and 28 patients of the old age group — Open circles Old age group Inner ring — Before operation Outer ring — After operation) Filled circles Young age group (Inner ring — Before operation Outer ring — After operation) Arrows indicate the average orientation consistent with the plots on each ring and the bracketed range on their either side the approximate ± S D interval The open diamonds indicate the limits of normal range and the filled diamond the average normal value (see text)

Comments on rhythm

The occurrence of pulmonary hypertension and/or pulmonary vascular disease in patients with preoperative atrial fibrillation was five out of 11 cases and that of systemic hypertension likewise five cases The pulmonary vascular resistance index was 258 dynes sec

cm⁻² (S.D 161) in the atrial fibrillation group surpassing significantly that in the old age group (*) and that of the entire series (** *) In respect of magnitude of the left-to-right shunt the patients with atrial fibrillation did not differ from the series as a whole but the shunt was larger on the average in the old age group than in the young age group the average Q_p/Q ratios being 3 54 (S D 1 57) 3 55 (S.D 1 26) and 3 27 (S.D 1 31) respectively The relative heart volumes averaged preoperatively in the group with atrial fibrillation 963 ml/m² *BSA* (S D 183) and postoperatively in the patients who were converted to sinus rhythm 595 ml/m² *BSA* (S D 95) Both figures exceed the corresponding values found for the age group of 40 years or older but not at any statistically significant level

The prolongation of atrioventricular conduction time was not related to any one of the haemodynamic characteristics nor to the radiological findings Complete atrioventricular block is not common in combination with ASD of secundum type Nakamura and Nadas (1964) reported 15 cases of congenital heart disease with complete heart block in three of which ASD secundum was concerned Two such ca es have recently been reported by Lev et al (1967) who concluded in their survey of aetiol ogy that the atrial septal defect was partly responsible for the a-v nodal changes In the case in the present series the patient s sibs and father had finger anomalies and congenital anomalies of the heart or major vessels occurred among the sibs

Occurrence of right bundle branch block pattern was to some extent related to the heart volume The relative preoperative heart volume averaged 665 ml/m² *BSA* (S D 210) in the patients with QRS prolongation of Grade 3 and 508 ml/m² *BSA* (S D 112) in tho e with Grade 2 the difference being significant (***) QRS prolongation of Grade 1 was associated with nearly equal heart volume as Grade 2 namely 524 ml/m *BSA* The patients displaying postoperatively shortening of QRS likewise had a significantly (***) larger heart volume (716 ml/m² *BSA* S D 194) preoperatively than the rest No correlation was established between the degree of intraventricular conduction delay and the Q_p/Q ratio or the right ventricular peak systolic pressure

ATRIAL ELECTROCARDIOGRAM

Morphology

P (II) was preoperatively bifid in 30 % of the patients of the young age group and in 50 % of the old age group it was tent shaped with central peak in 12 % of the young and about 30 % of the old group The first mode was mostly higher in the P waves with bifid shape preponderance of the second mode was comparatively rare No significantly different percentages of the different shapes were noted postoperatively except for the fact that the first mode in bifid P waves was not higher than the second mode as often as preoperatively

P (V₁) displayed preoperatively +— +0 ++ and —— pattern in 57 % 23 % 13 % and 4 % respectively and other combinations in 9 % The +— pattern was still postoperatively found in 37 % the +0 pattern in 2 % the ++ pattern in 10 % and the —— and —+ patterns in 33 % and 10 % respectively The —— type often presented a narrow but prominent positive deflection between the initial and terminal negative components of P (V₁) The terminal component was preoperatively positive or isoelectric in 34 % and postoperatively in 33 % The initial component was negative or isoelectric in 7 % preoperatively and in 46 % postoperatively There was no statistically significant difference between the groups of patients younger than 40 years and 40 years or older

Mean vector and atrial component vectors in the frontal plane

A P (Fig 6a) was preoperatively normal in 76 % and postoperatively in 68 % of the cases Right axis deviation was preoperatively noted in 10 patients (8 %) without any difference between the age groups and postoperatively in one single case Left axis deviation was preoperatively present in 16 % of the younger and 29 % of the older patients the correspond ing postoperative percentages being 34 % and 18 % A P averaged 41 4 degrees (S.D 25.2) and 48 4 degrees (S.D 17.2) in the younger and older group respectively before surgery The pos operative averages were 32 9 degrees (S D 28 7) and 36 4 degrees (S.D 17 7) with-

out any statistically significant difference between the age groups The change attendant on operation was statistically significant (*) in the group of patients younger than 40 years

$A\,P_{RA}$ (Fig 6c) was preoperatively normal in 97 % and postoperatively in 86 % Deviation to the right was preoperatively noted in four cases (3 %) but none to the left Postoperatively deviation to the right occurred in two patients only and deviation to the left in 14 patients (12 %) the proportionally greater part of which were younger than 40 years The preoperative $A\,P_{RA}$ averages of the young and old groups were 50 8 degrees (S D 24 9) and 59 0 degrees (S D 23 7) and the postoperative ones 41 2 degrees (S D 34 6) and 51 0 degrees (S D 29 7) No statistically significant differences were established between the groups In the young age group the change attendant on operation was statistically significant (*)

$A\,P_{LA}$ (Fig 6d) was normal in 89 % preoperatively and in 80 % postoperatively Preoperatively there was deviation to the right in one case and to the left in 12 (10 %) postoperatively only deviation to the left occurred in altogether 23 cases (20 %) Only slight differences between the age groups were noted The preoperative averages of the younger and older group were 30 3 degrees (S.D 26 0) and 34 4 degrees (S D 23 8) and those found after the operation 22 9 degrees (S D 30 0) and 25 2 degrees (S D 25 8) There were no statistically significant differences between the age groups or changes attendant on operation

Time and amplitude relations

P (II) duration was preoperatively normal in 97 out of 117 patients with sinus rhythm (in 83 %) It exceeded 120 msec in 20 patients 16 of them younger than 40 years (16 % of this age group) and four of them belonging to the older group (24 %) The postoperative P (II) duration was normal in 111 out of 118 patients with sinus rhythm exceeding 120 msec only in one patient of the old and in six of the young age group It was unchanged from the preoperative value in 27 % while it had increased in 15 % and decreased in 58 % The P (II) duration averaged preoperatively in the young and old age groups 99 8 msec (range 70—140) and 101 2 msec (range 60—140) and postoperatively 91 6 msec (range 60—110) and 89 3 msec (range 60—140)

P (II) upstroke time was preoperatively normal in 86 out of 117 patients with sinus rhythm (in 74 %) in nearly equal proportion in both age groups The length of the upstroke time was found to depend on which of the modes constituted the vertex of the wave There was never any prolongation if the first mode was preponderant In nearly half the cases presenting prolonged upstroke a central tent-shaped peak was observed the rest displayed a bi modal wave with a second mode of more or less greater height but markedly lower than the central type The upstroke times averaged preoperatively 39 8 msec (S D 11 7) in the young age group and 50 0 msec (S D 10 5) in the old age group The upstroke time in the entire series was postoperatively 36 4 msec (S D 8 5) and it was prolonged in only 5 % of the cases

P (II) amplitude was preoperatively normal in 111 out of 117 patients with sinus rhythm (in 95 %) and postoperatively in all patients It was preoperatively in excess of 0 2 mV in five patients younger than 40 years and in one patient of the old age group postoperatively the value was unchanged in 12 cases while it had slightly increased in 14 and decreased in the rest The preoperative P (II) amplitudes averaged in the group of patients younger than 40 years and in the old age group 0 12 mV (range 0 02—0 30) and 0 13 mV (range 0 03—0 22) and postoperatively 0 08 mV (range 0 06—0 20) and 0 07 mV (range 0 03—0 13)

P (V) initial force (Fig 7) was normal in 80 % of the cases preoperatively and in 72 % postoperatively and there was no significant difference between the age groups Only four patients presented preoperatively abnormally low initial force The force was abnormally high in 19 patients younger than 40 years and in one patient of the old age group that is in 16 % of all cases Abnormally high initial force occurred postoperatively in six cases only while 27 cases had an abnormally low force without any statistically significant difference between the age groups either The initial force averaged in the group of patients younger than 40 years and in that of older patients +0 050 mmsec (S D 0 025) and +0 027 mmsec (S D 0 016) preoperatively and +0 009 mmsec (S D 0 014) and —0 006 mmsec (S D 0 012) postoperatively the change being signif-

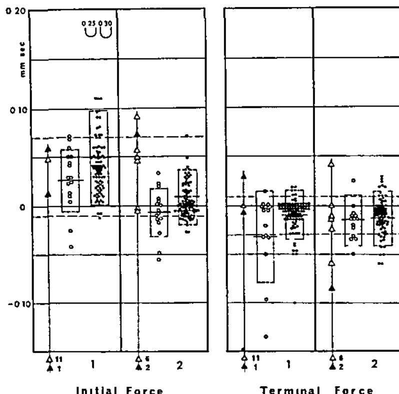


Fig 7 Initial and terminal P(V_1) forces before (1) and after (2) operation in 98 patients of the young age group (●) and 28 patients of the old age group (o) — The ± 2 S.D range of each group has been bracketed and its mean indicated by a heavy cross line The shaded belts indicate the normal ranges of variation (± 2 S D) Cases with preoperative and/or postoperative absence of P wave have not been included in the main plots they are separately represented by triangles (▲ young △ old age group) mounted on a vertical line those with absence of P wave at the particular examination (1 or 2 respectively) being jointly represented by one symbol in the lower margin of the figure and their number inscribed beside this symbol

icant (***) in both age groups Those with preoperative atrial fibrillation but postoperative sinus rhythm displayed clearly higher initial force on the average after surgery than the other patients in the same age group The respective means were all within the normal limits of variation

P (V_1) terminal force was preoperatively normal in 77 % and postoperatively in 64 % of the cases with no difference between the age groups in this respect Preoperatively the terminal force of ten patients younger than 40 years and of one older patient was abnormally positive while abnormal negative values were found for ten younger and six older subjects (10 % and 35 % of the respective group) that is in altogether 12 % of the patients Postoperatively abnormally high positive terminal force occurred in 14 younger and three older patients and abnormally negative terminal force in 21 younger and six older patients (21 % and 27 % respectively) The terminal forces of the young and of the old age group averaged preoperatively —0 010 mmsec (S D 0 013) and —0 032 mmsec (S D 0 024) and postoperatively —0 013 mmsec (S.D 0 014) and —0 015 mmsec (S D 0 013) Whenever preoperative atrial fibrillation had given place to postoperative sinus rhythm terminal forces on the same level as in the rest of the series were found The means are all within normal range except for that of the patients aged 40 years or older which was abnormally negative before the operation but not after surgery ($t = 1\ 25$ $P<0\ 20$)

Table 9 Number of cases with right and left atrial overload before and after surgery in two age groups according to fulfilment of 1 2 or 3 items of the criteria Total 117 cases (100 younger and 17 older patients) of ASD secundum in sinus rhythm before surgery and 118 cases (96 younger and 22 older patients) after surgery in sinus rhythm 3 cases with residual shunt excluded

Criteria Number of items fulfilled	Right atrial overload				Left atrial overload			
	Age < 40		Age ≧ 40		Age < 40		Age ≧ 40	
	before	after	before	after	before	after	before	after
1	19	12	3	1	27	33	3	7
2	5	—	1	—	10	10	2	3
3	2	—	—	—	—	6	2	—
Total	26	12	4	1	37	49	7	10
%	*26*	*12*	*24*	*6*	*37*	*51*	*32*	*45*

Atrial fibrillatory waves — The amplitude of fibrillatory waves was 0 1 mV or less in eight out of 12 cases exceeding this value in four A record obtained during sinus rhythm was also available in seven of these twelve cases The terminal P(V_1) forces were abnormally negative in two of them and in both cases the fibrillatory waves also exceeded the amplitude of 0 1 mV Operation revealed in one of these two cases exceptionally thick atrial walls with heavy muscular trabeculation

Atrial overloading The criteria of atrial overloading were applied to the findings presented in the foregoing Table 9 shows the incidences of RAO and LAO in the different groups Considering the cases in which more than one item of the criteria was fulfilled

Table 10 QRS (V_1) morphology in 129 cases of ASD secundum (101 patients younger than 40 years and 28 patients aged 40 years or older) The preoperative and postoperative distributions of the Q, R S R and S deflections, each in three voltage categories (≧ 0 5 mV, < 0 5 mV, and absent) are stated as percent of all cases of the age group

Deflection		Age < 40 years					Age ≧ 40 years				
≧ 0 5 mV	2°	Q	R	S	R	S	Q	R	S	R	S
< 0 5 mV	1°	q	r	s	r'	s	q	r	s	r	s
absent	0°	—	—	—	—	—	—	—	—	—	—
Before operation	2°	0	15	24	42	7	0	4	14	46	0
	1°	12	85	59	28	14	4	92	64	43	18
	0°	88	0	17	30	79	96	4	22	11	82
After operation	2°	0	13	64	13	1	0	7	46	36	0
	1°	7	87	32	44	15	7	93	43	32	4
	0°	93	0	4	43	84	93	0	11	32	96

RAO occurred preoperatively in 7 % of all cases but not at all postoperatively LAO was thus established preoperatively in 12 % and postoperatively in 16 % of the cases The differences between the age groups were insignificant At least one item indicating atrial overloading right or left was established preoperatively in 67 cases and postoperatively in 69 (57 % and 58 % respectively) The cases with preoperative existence of two or several items number 22 preoperatively and 19 postoperatively (19 % and 16 % respectively) It is to be noted that if one single item is deemed sufficient for diagnosis of atrial overloading there were seven preoperative and three postoperative instances of combined left and right atrial overloading

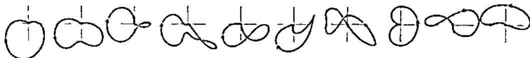
Comments on atrial electrocardiograms

The radiological enlargement of the atria, as assessed from the lateral radiogram had no relation to the findings made from the atrial electrocardiogram atrial enlargement being equally frequent in the patients with and without atrial overloading pattern discernible in the electrocardiogram The P wave changes too were unrelated to the magnitude of the *a* wave in the intracardiac pressure recordings to the magnitude of the left-to-right shunt and to presence of systemic arterial hypertension The considerable prevalence of preoperative LAO may be related to the fact that during atrial systole a marked increase occurs in the left-to-right shunt across the defect as was recently shown by Levin et al (1968) In half of the cases with abnormally high P(II) amplitude pulmonary hypertension was present and the upstroke time was at the upper limit of normal in the group of patients aged 40 years or older who had on the average a slightly smaller shunt and higher pulmonary vascular resistance index than those of the younger age group

The atrial haemodynamics were profoundly changed by the operation The large left-to right shunt was postoperatively absent the left atrial or pulmonary wedge pressure remained on the same level or was slightly elevated whereas the right atrial pressure was distinct ly lower than preoperatively In the radiogram the heart in particular its right cavities was smaller after surgery The following simultaneous changes were noted in the electrocardiogram marked decrease in the prevalence of right atrial overload pattern increase in that of left atrial overload pattern and some shortening of the duration of atrial activation

The morphology of the left atrial wave component displayed rather lesser postoperative changes in that there was no change in the prevalence of terminal negativity or isoelectricity in $P(V_1)$ in contrast to the common and often profound changes in the initial part of $P(V_1)$ These changes may probably be partly interpreted in terms of the regional potential variation concept since in many cases considerable reduction in size of the heart may be considered to bring the left atrial free wall into closer proximity of the electrode of the V_1 lead Moreover the direct traumatizing effect on the right atrial wall interatrial septum and Eustachian ridge produced by the surgical interference may result in abnormal spread of activation in the muscular walls and in the internodal or interatrial pathways The functional consequences of such anatomical changes are hardly known (James 1963) but they might cause aberrant activation of both atria This has particular bearing on the right atrial component of the P forces The occasionally seen inversion of the terminal P(V) force from preoperative negativity to positive deflection after surgery may have the same traumatic origin seeing that the change in the anatomical axis of the heart is not able to account for the phenomenon in the cases in question Haywood and Selvester (1966) found in about one-third of a series of normal atrial vectorcardiograms a third central component which they attributed to septal activation The central positive peak in an otherwise negative P(V) in some postoperative instances might likewise be related to abnormal septal activation Similarly the preoperatively common bifid P(II) of normal duration might be explained in part on the basis of abnormal activation of the defective atrial septum

All the above explanations considered such random lesions are still unable to account for the rather prevalent though not very remarkable trend of diminishing right atrial forces and increasing left atrial forces in the series as a whole It would thus seem reasonable to ascribe them largely to the changes which take place in atrial haemodynamics



<40	9	29	23	20	11	1	5	–	2	1	101
≧40	3	4	11	4	3	2	–	1	–	–	28
ALL	12	33	34	24	14	3	5	1	2	1	129
%	93	256	264	186	108	23	39	08	1.5	08	1000

Fig 8 Distribution of 101 patients of the young age group and 28 of the old age group according to type of frontal plane QRS sE loop recorded before the operation — The loops were constructed from their electrocardiograms using a corrected hexaxial frame (see text)

VENTRICULAR ELECTROCARDIOGRAM

Morphology

Preoperatively the *QRS complex* was normal (rS) in 5 % of the patients younger than 40 years and in 4 % of the older group while the corresponding postoperative percentages were 23 % and 14 % The three commonest QRS morphologies in V_1 were for the different age groups and in the order of decreasing prevalence within them preoperatively in the young group rsR rsr' rs likewise in the old group rsR rsr' rR postoperatively in the young group rSr rS rsr in the old group rsR rsr rS The shape of the QRS complex in V_1 was subjected to analysis as described before The incidences at which each type of deflection (large small or absent) occurred in both age groups before and after surgery are seen in Table 10 (p 60) The table reveals that the combinations enumerated above were commonest preoperatively The distribution of r or R was highly stable and unaffected by the patient s age and by the surgery S occurred slightly more frequently in the group of patients younger than 40 years R was equally common in both age groups preoperatively but postoperatively its prevalence in the old age group was three times that in the young patients Absence of r or R was commoner in the young group As a rule the deflections were larger in the young than in the old age group The cases with residual shunt displayed unaltered morphology or one in which rSr had been replaced by rsR

T wave negativity in the chest leads was common preoperatively No attempt was made to differentiate between primary and secondary T wave inversions Normal T waves in V_1—V_6 were seen in 46 % and T waves more strongly negative than 0 5 mV in 6 % of the patients younger than 40 years In the old group the percentage of normal T waves was only 25 % and that of strongly (more than 0 5 mV) negative T waves was 14 % Postoperatively, the occurrence of normal precordial T waves amounted to 73 % and that of persisting deep negative T waves to 4 %, in the young group the corresponding values for the old group were 46 % and 11 % T inversions extending up to V_6 were preoperatively noted in 12 % of the young and 28 % of the old group and postoperatively in 6 % and 4 %

Mean QRS vector in the frontal plane

A QRS (Fig 6 b) was preoperatively normal in 23 % of the young age group and in 14 % of the old age group the respective postoperative percentages being 69 % and 61 % Values between +30 and —90 degrees were preoperatively recorded in 5 % and 4 % but postoperatively in as much as 14 % and 11 % of the younger and older patients respectively Positions between +95 and —90 degrees were preoperatively found in 72 % and 82 % of the young and old age group respectively and postoperatively in 17 % and 28 % Left axis deviation proper (A QRS between —30 and —90 degrees) was preoperatively seen in three cases and postoperatively in four The orientation of A QRS was preoperatively + 104 8 degrees (S D 38 0) in the young and + 118 2 degrees (S D 35 2) in the old group Postoperatively it was + 61 2 degrees (S.D 38 8) and + 70 4 degrees (S D 41 9) respectively In both age groups a statistically significant shift (* * *) of the mean frontal axis to the left had ensued on operation No statistically significant differences were established between the age groups

Time and amplitude relations

The *QRS duration* has been considered above in connection with the disturbances of conduction As has been mentioned it equalled or exceeded 120 msec preoperatively in 17 % of the patients younger than 40 years and in 32 % of the old age group while the respective postoperative percentages were 4 % and 17 %

The *QR/QRS (V_1) ratio* averaged in the total series 0 61 preoperatively and 0 63 (range 0 14—0 89) postoperatively It was less than 0 60 in 44 patients before and in 35 after surgery (35 % and 28 % respectively) No statistically significant differences were established between the age groups

The S *($V_{5,6}$) duration* was 46 7 msec (S D 46 8) in the group of patients younger than 40 years and 58 0 msec (S D 21 3) in the old age group the respective postoperative values being 28 5 msec (S D 37 0) and 40 9 msec (S D 18 7) The postoperative values of the groups differ significantly (***) and the change due to operation was also statistically significant (*) in both groups There were only eight instances in which the duration of S ($V_{5,6}$) was the same or had increased after surgery both age groups presenting four such cases S ($V_{5,6}$) was preoperatively absent in two patients and postoperatively in three The cases with residual shunt displayed reduction in duration of S ($V_{5,6}$) by 5—10 msec after operation

The *R or R (V_1) amplitude* in the group of patients younger than 40 years and in the old group was preoperatively 0 64 mV (S D 0 52) and 0 52 mV (S D 0 40) respectively and after surgery it was 0 36 mV (S.D 0 24) and 0 40 mV (S.D 0 28) respectively There were no statistically significant differences between the groups while in the young group the amplitude had diminished significantly (***) on operation Unchanged amplitude of R or R (V) was postoperatively noted in 25 instances (20 % of all patients) and increased amplitude in 16 (13 %) In the cases with residual shunt reduced unchanged or increased amplitude was found independent of the result of surgery R or R (V) amplitude in excess of 1 5 mV combined with QRS duration equalling or exceeding 120 msec or in excess of 1 0 mV in combination with QRS duration less than 120 msec was preoperatively encountered in 11 patients of the group under 40 years of age and in two older patients (11 % and 7 % of the respective group) and in one patient of either group postoperatively

The *R/S or R/S (V_1) ratio* was in excess of 1 0 preoperatively with simultaneous R amplitude of 0 5 mV of higher and QRS duration less than 120 msec in 35 patients of the young and eight of the old age group (36 % and 29 % respectively) postoperatively this condition was found in eight and four patients (8 % and 14 %) respectively

The *R ($V_{5,6}$) amplitude* in the groups of young and old patients was preoperatively 1 33 mV (S D 0 57) and 1 22 mV (S D 0 61) respectively the corresponding postoperative values being 1 48 mV (S D 0 56) and 1 62 mV (S D 0 77) No statistically significant differences were established between the groups The increase in amplitude attendant on operation in the group of older patients amounts to a statistically significant change (*)

Right ventricular diastolic overloading — The criteria of right ventricular diastolic overloading were preoperatively fulfilled by 28 % and postoperatively by only 4 % of the patients younger than 40 years and correspondingly by 36 % and 14 % of those aged 40 years or older Taking into account also the cases presenting complete right bundle branch block the re pective figures are 45 % and 7 % for the young and 68 % and 32 % for the old age group

Right ventricular systolic overloading — The criteria of right ventricular systolic overloading were applied to the findings reported in the foregoing and they were found to be fulfilled in the young age group preoperatively in 43 cases (44 %) and postoperatively in 19 only (19 %) the corresponding figures in the old age group were nine cases (32 %) and five cases (18 %) respectively

In respect of *left ventricular systolic overloading* the criteria relating to voltage were fulfilled by two of the younger patients preoperatively and by three postoperatively in the old age group this condition was found in one patient before and in two after surgery

QRS sÊ frontal loop

The *QRS sÊ frontal loops* were plotted in all cases both preoperatively and postoperatively Fig 8 (p 62) displays the preoperative distribution of ten different types of loop The second and third type were most *frequently encountered* the former being more common among the younger and the latter among the older patients Loops with counterclockwise inscripton only were found in nine instances (7 %), only three of them (2 3 % of all ca es) having their loop in the 3rd and 4th quadrants of the frontal frame The scalar electrocardiograms obtained in these cases were equal to those of the rest of the series except that left axis deviation was present in all of them Of two patients with WPW syndrome one had a counterclockwise figure-eight loop (type 7) while the other had a clockwise loop The sole remarkable change observed after operation was usually considerable reduction in size of the centripetal part of the loop in the 2nd and 3rd quadrants

Comments on ventricular electrocardiogram in relation to haemodynamic findings

In 58 cases subjected to more detailed haemodynamic analysis a study was made of the relationships which might exist between the preoperative R/S or R'/S (V₁) ratio and the R or R' (V) amplitude on one hand and on the other hand the right ventricular peak systolic pressure (P_{sr}) stroke volume index (SVI_s) stroke work index (SWI_{sr}) pressure-time index (PTI_{sr}) mean systolic ejection rate ($MSER_{sr}$) pulmonary-to systemic flow ratio ($\dot{Q}_p/Q$) pulmonary vascular resistance index (R_p) and pulmonary to systemic resistance ratio (R_p/R) On the whole poor correlation between the R/S ratio or the R voltage in the V lead and the variables enumerated above was noted This is in accordance with the observations of several authors discussed in Chapter II

No correlation on these electrogardiographic characteristics of systolic right ventricular overloading was established for the flow parameters $\dot{Q}_p/Q$ $MSER_{sr}$ and SWI_{sr} while SVI_{sr} displayed slight negative correlation on them Of the pressure parameters P_{sr}, R_p/R R_p, and PTI_{sr} were found to present slight positive correlation with the amplitude of R(V_1) when this amplitude was in excess of 1 mV, and a somewhat stronger correlation with R/S (V_1) Very poor correlations with P_{sr} were noted while the correlation with R_p/R_s was slightly stronger Thus of the cases with R_p/R_s equal to 0 1 or less R/S (V_1) was higher than 1 0 in 22 % and the R (V_1) or R' (V_1) amplitude was abnormally high in 6 % the corresponding percentages for cases with R_p/R_s in excess of 0 1 being 83 % and 33 %, respectively Most notable though not very strong either was the correlation of electrocardiographic signs of right ventricular overloading with PTI_{sr}, consecutive increments of PTI_{sr} upwards of 10 mm Hg by steps of 5 mm Hg involving increase of the percentage of cases with R/S higher than 1 0 from 16 % to 27 % to 60 % and to 100 % Table 11 contains some rough data illustrating the correlations between PTI_{sr} SVI_{sr} and the R/S (V_1) ratio these data suggest that when volume and pressure load occur in combination the latter is more important as determinant of the R or R' voltage in lead V_1

The RSR' pattern in the V_1 lead constituting a sign of right ventricular diastolic overloading was considered in conjuction with the flow parameters and radiological findings On the average cases in which the criteria of diastolic overload were fulfilled presented larger heart volume slight though not consistent positive correlation with the right ventricular flow parameters was also noted Similar observations were made in respect of the duration of S (V_{s}) even though it is a fact that shortening of this time interval proved to be the most constant postoperative change

The shape of the frontal QRS loop was not related to the haemodynamic or radiological findings although it is noted that pulmonary hypertension was only present in ca es presenting loops of type 3 or 4 in agreement with Lee and Scherlis (1966) vectorcardiographic observations The cases in which the loop was inscribed counterclockwise and predominantly located in the 3rd and 4th quadrants yielded operative findings typical of ASD secundum they presented no different clinical or haemo dynamic picture from that in the rest of the series

Table 11 Relations between right ventricular pressure time index (PTI_{rv}), stroke volume index (SVI_{rv}) and number of cases with R/S or R/S (V) > 1 0 when R or R $\leqq$ 0 5 mV and QRS duration < 120 msec When PTI_{rv} is in excess of 10 mmHg sec, the peak systolic ventricular pressure is usually over 40 mmHg When SVI_{rv} is in excess of 100 ml/m²BSA the pulmonary-to-systemic flow ratio is usually over 2 0

		PTI_{rv} < 10 0 mmHg sec			PTI_{rv} $\geqq$ 10 0 mmHg sec	
SVI_{rv} < 100 ml/m²BSA	Cases	7		Cases	14	
	R/S > 1 0	—	(0 %)	R/S > 1 0	8	(57 %)
SVI_{rv} $\geqq$ 100 ml/m²BSA	Cases	12		Cases	21	
	R/S > 1 0	3	(25 %)	R/S > 1 0	4	(19 %)

GENERAL COMMENTS ON ELECTROCARDIOGRAPHIC FINDINGS

The electrocardiographic findings in ASD secundum reported in the literature are somewhat inconsistent as regards atrioventricular conduction the atrial electrocardiogram and the correlation of electrocardiographic findings on haemodynamic findings On the other hand as has been said before rather general agreement exists concerning the characteristic features of QRS morphology and of those in the vectorcardiographic pattern in the condition in question

On the supraventricular level disturbances of conduction and of rhythm seem to be not uncommon in ASD secundum as witnessed by the prevalence of atrial fibrillation (Wood 1962) and by the continuous increase in incidence of arrhythmias with increasing age reported in Chapter V In different series in the literature atrioventricular conduction has been found to be retarded in 5—25 % of the cases (Bedford et al 1941 Limon et al 1953 Campbell et al 1957 Lee and Scherlis 1962 Derra et al 1965 Gault et al 1968 Petersson 1967) or it has been within normal limits (Walker et al 1956) In the present series prolonged P-R time was noted preoperatively in 8 % and postoperatively in 6 % but on the average this time was normal and unaffected by surgery However sinoatrial conduction deficiency was preoperatively established in one patient while in the electrocardiogram of another patient who had sustained atrial tachycardia with 2 1 A V block no signs of sinoatrial conduction could be detected during the short spells of absence of his arrhythmia Moreover several cases displayed preoperatively some evidence of intraatrial and interatrial conduction delay or aberration Sinus rhythm was absent from the atria in 9 % Thus ASD secundum often seems to be associated with various supraventricular disturbances of rhythm and conduction and this is more common in the group of patients aged 40 years or older On the other hand intraatrial and interatrial conduction and activation abnormalities were produced by the operation in a considerable number of cases but this does not change the fact that sinus rhythm was retained more commonly after than before surgery perhaps constituting a sign of improved atrial function

The same is also true in respect of ventricular conduction as is evident from the substantial reduction in number after surgery of cases with complete right bundle branch block pattern and from the almost regular change towards normal of QRS and T in the precordial leads The sole potential in V_1 not affected by age nor by surgery was the initial r deflection due to the vectors generated by activation of the left septal mass The most constantly occurring postoperative sign of right ventricular involution was narrowing of S (V_6) which failed to materialize in 6 % only The natural fact that the right ventricular involution is time-dependent is borne out by the observation that intraventricular conduction delay of grade 3 was three times as frequent among the patients followed for less than 30 months as in those with longer follow-up period and likewise by the circumstance that especially in older patients the R or R (V_1) amplitude was significantly lower (**) in patients followed for a period longer than 30 months than in

those with shorter follow-up period This conforms to the findings of Dreifus et al (1959), Loogen et al (1961) and Reindell et al (1962)

Only few reports can be found in the literature concerning correlations between the atrial electrocardiogram and haemodynamic findings in ASD secundum High P(II) has been noted in 5—40 % of the cases and P(II) has often been found to be broad and/or notched (Bedford et al 1941, Walker et al 1956 Toscano Barboza et al 1958 Davies et al 1960 Massie and Walsh 1960, Derra et al 1962 Lee and Scherlis 1962 Sánchez-Cascos et al 1963 Markman et al 1965 Cohn et al 1967 Petersson 1967) In the V_1 lead the P wave is of + — or +0 type in 50—65 % and of 0— type in 3 % All these findings have been attributed to abnormal atrial haemodynamics Some authors who studied the atrial vectorcardiogram did not find any correlations between the morphology and/or dimensions of the P wave and haemodynamic findings (DeOliveira et al 1962 Reindell et al 1962) Others again attribute the occurrence of large P waves either to large shunt volume (e g Kjellberg et al 1959) or to elevated pulmonary pressure (e g Barber et al 1950 Sanchez Cascos et al 1963) Sanchez-Cascos et al were the only ones who found a mathematical relationship between P (II) duration and/or upstroke time and shunt volume they found similar relations also between P (V_1) morphology and left atrial systolic pressure No mathematical relationships were found in the present study between the atrial pressure or flow parameters and the P wave vectorial characteristics time relations amplitudes or morphology in the II and V_1 leads other than association of abnormally high P (II) with pulmonary hypertension However there was distinctly decreased postoperative prevalence of right atrial overload pattern and some increase of that of left atrial overload pattern which can be attributed to corresponding changes in atrial haemodynamics

Poor correlations were established as a rule between ventricular electrocardiographic overload pattern and the haemodynamic findings in the present series Even though correlation of this kind was present individual cases showed a high degree of inconsistency in this respect This conforms to earlier reports (Walker et al 1956 Campbell et al 1957 Besterman 1962 Reindell et al 1962 Zaver and Nadas 1965 Petersson 1967) Common occurrence of R in cases with large shunt has been emphasized by several authors (e g Davies et al 1960 Lee and Scherlis 1962 Petersson 1967), this was also apparent in the present series in which however, numerous exceptions from the rule rendered the overall correlation quite poor In all likelihood this is partly due to diminishing of the shunt when the pulmonary pressure begins to rise and the right ventricle begins to fail In fact the R (V_1) amplitude showed stronger correlation on PTI_{sr} than on SVI_{sr} Likewise negative T waves occurring in the chest leads became more prevalent with increasing age and heart volume, as also Davidsen (1960) Campbell et al (1957) and Reindell et al (1962) have reported However their occurrence was not consistently correlated with haemodynamics except for their frequent disappearance and general abatement after surgery

The electrocardiogram does not seem to be any sensitive means for discriminating cases with residual shunt seeing that the changes present in it may persist in spite of successful surgery However postoperative normalization of the electrocardiogram strongly speaks against presence of a residual shunt as Loogen et al (1962) have pointed out The same discriminative power may perhaps be ascribed to remarkable narrowing of S (V_{6}) The electrocardiogram is certainly of value in selecting patients for surgery because presence of the right ventricular systolic overload pattern strongly suggests pulmonary vascular disease although it is true that false positive findings are common as also stated by Roman et al (1961) and by Scott (1967) Left axis deviation was noted in three cases of the present series and the frontal axis had preoperatively a direction to the left of 0 degrees in five cases The cases with left axis deviation also invariably presented a frontal QRS loop indistinguishable from those found by the same method in primum defects (Perasalo et al 1964) In ASD secundum left axis deviation has been found in 0—11 % (Pryor et al 1959 Davidsen 1960 Sellers et al 1966 Cohn et al 1967 Wolf et al 1968) The kind of frontal loop typical of secundum defect is rather less common in primum defects for which reason the electrocardiogram possesses value in discriminating primum defects from secundum defects (Burchell et al 1960)

SYNOPSIS

The electrocardiographic records made before and after operation in 129 cases of ASD secundum were analyzed in regard of the characteristics of conduction rhythm atrial complex and ventricular complex

Sinus rhythm was preoperatively present in 99 % of the patients younger than 40 years and in 61 % of the group of older patients Atrial fibrillation was preoperatively present in 11 cases ten of them being patients aged 40 years or older and postoperatively it occurred in four patients of this same age group

Minor atrial conduction defects were found both before and after operation The P—R time was normal both preoperatively and postoperatively on the average It was prolonged in 8 % of the cases before and in 6 % after surgery The intraventricular conduction was preoperatively retarded in all cases the QRS duration was 120 msec or longer in 17 % of the patients younger than 40 years and in 32 % of the older group The corresponding postoperative percentages were 4 % and 17 % respectively

Right atrial overload pattern was preoperatively noted in 7 % of the young and 6 % of the old age group while there was no such case postoperatively Left atrial overload pattern was preoperatively present in 10 % of the young and 22 % of the old age group and postoperatively in 16 % and 13 % respectively

Abnormally high P (II) amplitude occurred preoperatively in eight cases only and postoperatively in none The atrial mean frontal vectors and their right and left atrial components had moved slightly leftwards after surgery The P (V_1) initial force was pre operatively normal on the average but it was still markedly reduced after operation The P (V_1) terminal force was abnormally negative preoperatively in the old group but normalized postoperatively

The commonest preoperative QRS (V_1) morphologies were rsR rsr rs in the younger and rsR rsr rR in the older patients S deepened postoperatively and R diminished particularly in the younger patients QRS was normal in six patients before and in 26 after operation its normality was commoner among the patients younger than 40 years The most constant change associated with the operation was narrowing of S (V_{6}) which failed to occur in 6 % only Left axis deviation and counterclockwise frontal QRS sE loop were found in three cases The mean frontal QRS moved about 50 degrees leftward on the average

There was poor correlation between the electrocardiographic and haemodynamic findings Apart from the changes connected with abolishment of the shunt by surgery only association of high P (II) and high R/S R /S R or R in the V lead with pulmonary hyper tension (i e high right ventricular pressure-time index and high pulmonary-to-systemic resistance ratio) was found

VIII RADIOLOGY

METHODS

Technique

All radiograms were taken in the course of routine examination at the radiological department of the hospital with 150 cm tube film distance in nearly all cases in postero-anterior as well as left lateral projection in erect posture after moderate inspirium without Valsalva s manoeuvre and without synchronisation of the exposure with ECG Some of the preoperative radiograms however were taken with 175 or 200 cm tube-film distance Tomographic examination of the central and basal lung fields was performed in all cases in addition to the above ordinary radiography in order to visualize the pulmonary vessels

Measurements and interpretation

Each patient s latest preoperative radiogram and the radiogram made at follow up were subjected to analysis in this study The magnification was standardized by applying an individual correction factor to all dimensions measured from each radiogram The factor was obtained by measuring the greatest transverse diameter of the thoracic inlet (i e the greatest transverse distance of the inner contours of the first ribs) in the postoperative and preoperative postero anterior radiograms and calculating their ratio by which all dimensions measured from the preoperative film were multiplied before subjecting them to further calculations This was only omitted in such cases in which the patient had grown in height since the operation

The *heart volume* was calculated by the ellipsoid approximation method originally based on the investigations of Rohrer (1916) Liljestrand et al (1939) and Jonsell (1939) and further improved e g by Kjellberg et al (1949) and by Musshoff and Reindell (1957) The outline of the heart shadow in the p-a film was completed to an ellipse by drawing and the perpendicular long and short axes (l and b respectively) of this ellipse as well as the greatest horizontal depth of the heart shadow in the lateral film (d) were measured The heart volume was then obtained by the formula $V = K \times k \times l \times b \times d$ where K equals 0 41 and k is the individual magnification correction factor The volume is stated in millilitres (ml) or in ml relative to body surface area in m² (ml/m² *BSA*) the latter being obtained from the nomogram presented by DuBois and DuBois (1916)

The *left ventricular prominence in the lateral view* was measured according to Hoffman and Rigler (1965) the distance was measured which the left ventricle extends posteriorly to the posterior border of the inferior vena cava at a point 2 cm cephalad to the crossing of vena cava and left ventricle This measurement was made in a plane extending posteriorly and parallelling the plane of the intervertebral space on this level, and the result was expressed in millimetres (mm)

Cross sectional area of the aortic arch relative to body surface area — The method employed in determining this characteristic will be described in full elsewhere (Siltanen in preparation) In short, the technique was as follows The outline of the aortic arch in the p-a radiogram was completed to a circle by drawing the radius of the circle equalling the smallest radius of curvature of the arch shadow The diameter of this hand drawn circle was measured in two perpendicular directions and if there was a difference the mean was taken for its diameter (d) expressed in centimetres (cm) The relative cross-sectional area was then calculated by the equation $A = \pi \times d^2/4 \times BSA$ Taking into account the individual correction for magnification, the final formula $A = 0\ 68 \times (k\ d)^2/BSA$ was obtained The values found are stated in square centimetres relative to m² of body surface are (cm²/m² *BSA*)

The *heart shape* was mainly analyzed according to the principles set forth by Reindell et al. (1962) and by Kleinpzig and Frisch (1965) All findings were evaluated by scoring from 0 to 2, zero score denoting normal finding

Right atrium — The following scoring was used to record the radiological appearance of the right atrium 1 — Right atrial arch in the p a radiogram extending upwards to the lower border of the right pulmonary artery 2 — The right atrial contour completed to a circle does not cross the left border of the spine but extends into the right lung field

Left atrium — The following scoring was used to record the radiological appearance of the left atrium 1 — Shallow oesophageal impression in the lateral view 2 — Marked impression in the oesophagus in

the lateral and/or p a radiogram left auricular appendage possibly visible and double arch contour of the right cardiac border in the p a view

Right ventricle — The following scoring was used to record the radiological appearance of the right ventricle 1 — Anterior cardiac border in the lateral view touching the dorsal contour of the sternum 2 — Anterior cardiac border on the dorsal contour of the sternum in the lateral view pulmonary conus visible in the p a view heart apex rounded and left cardiac border shallowly bulging.

Left ventricle — The following scoring was used to record the radiological appearence of the left ventricle 1 — Slight bulging of the left ventricle into retrocardial space in the lateral view 2 — Distinct bulging possibly superimposed on the contour of the left leaf of the diaphragm.

Pulmonary artery — The following scoring was used to record the radiological appearance of the pulmonary artery 1 — Slight enlargement 2 — Marked enlargement

Pulmonary plethora was assessed according to Fouche et al. (1963) with some simplifications The pulmonary vessels were examined for dilation separately in the upper middle and basal lung fields Presence of increased pulmonary vascular markings in any of these areas was scored with one point, whereby the maximum scoring was 3 Fluoroscopy was also performed in every case for detection of hilar dance if any

Chronic pulmonary interstitial oedema — Subtle signs of oedema were judged according to Steiner (1958) and Logue et al. (1953) as follows 1 — Increased pulmonary interstitial density with clouding and/or loss of sharp definition in vessels resulting in haziness in the lung bases 2 — Septal lines thickened fissures

Comments on methods

The ellipsoid approximation method of heart volume determination has good reproducibility with an error of 3–5% in double determinations and insignificant inter-observer error (e g Axen et al. 1946 Musshoff and Reindell 1957) and it is in good agreement with postmortally found displacement volumes of the heart and results of tomographic volume determinations (Friedman 1951 Gebhardt 1957) Larger errors may be incurred in cases in which the shape of the heart deviates much from that of an ellipsoid unless individual corrections for form are applied (Larsson and Kjellberg 1948) No attempt to apply such corrections was m de in the present study but the magnification factor 0 41 was used instead of 0 42 because throughout the series the $d^2/l \times b$ ratio was abnormally low indicating flat heart shape There is no doubt that in spite of this precaution the error was greater than encountered in cases presenting normal heart sape but it is to be noted that the heart volumes were mainly needed for comparison between the preoperative and postoperative conditions only

The tomographic findings are not treated apart from the findings relating to anomalous pulmonary venous drainage in this work.

The accuracy achieved in measuring the left ventricular inferior caval overlapping was checked by means of quintuplicate determinations in ten cases which suggested that the scatter of a repeated measurement depends on its magnitude and is at a maximum approximately consistent with the standard deviation and double standard deviation limits indicated in Fig 10 It is obvious that the dimension concerned here and others of the same type are highly sensitive to rotation of the heart, particularly in the postero anterior direction and such rotation is common in ASD after surgery as a result of the right ventricle s reduction in size The error introduced by rotation tends to lower the postoperative overlapping values. The normal value is 11.5 mm (S D 4 7) and it follows that values in excess of 21 mm may be considered abnormal. Placing the limit at 18 mm Hoffman and Rigler (1965) found false positive and ftlse negative indications in numbers equivalent to 11 % and 19 % respectively They could establish the crossing point, which is essential for the measurement, in 76 % of their cases In the present study the crossing point was found in every case

The relative cross sectional area of the aortic arch was determined in 100 normal individuals of both sexes (59 men and 41 women) aged 16 to 86 years who had no arterial hypertension apparent atherosclerosis or organic heart disease The area was found to depend on age conforming to the regression equation $y = 1\,81 + 0\,0475\,x$ where the independent variable (x) is the age in years and the dependent variable (y) is the relative cross sectional area of the aortic arch in cm /m BSA The corresponding regression line with its 95 % tolerance limits has been entered in Figs. 12a and b Previously Dotter and Steinberg (1949) found in their angiographic studies of normal individuals that the absolute diameter of the aorta increases with increasing age Arvidsson (1963) demonstrated by angiography in eleven normal children the existence of linear correlation between aortic size body surface area and age According to him, the descending aorta has a cross sectional area equalling $1\,5 \times BSA$ and the ascending aorta one of $3\,0 \times BSA$ while rof the latter the figure of $3\,16 \times BSA$ was found by Castellanos and Hernandez (1967) in 14 normal individuals whose ages averaged 10 8 years Arvidsson points out that the aortic diameter displays considerable fluctuation with the different phases of the heart cycle the measurements cited were therefore invariably made by means of exposures synchronized with end systole It is accordingly possible that in the present study the considerable scattering of the values may be largely due to omission to time the exposures In the literature no reports have been found concerning the aortic cross sectnal area relative to BSA in adults of different ages, nor any on the determination of this dimension from ordinary p a chest radiograms It is an old expedient for judging the size of the aorta in a p-a film to measure the distance of the left lateral border of the aortic arch from the midline of the spinal shadow This procedure is much

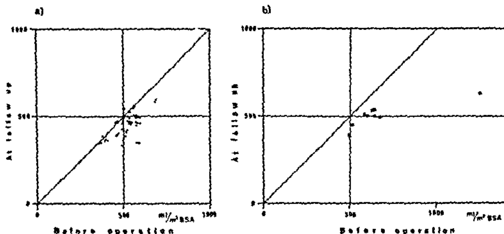


Fig 9 Relative heart volumes of 101 patients of the young age group (Fig 9 a) and 28 patients of the old age group (Fig 9 b) before operation and at follow up — *In cases represented by a plot lying under the diagonal line the heart volume has been reduced as a result of surgery*

applied especially in insurance medicine and in epidemiological studies but it constitutes a rather inexact estimation, which is affected by numerous factors other than the aortic diameter According to the N Y H A. Criteria Committee (1964) measurement of the aortic diameter from the impression caused by the aortic arch on the barium filled oesophagus has no clinical importance

Radiological shunt assessment was used in the original method of Fouche et al (1963) three lung fields in both lungs being separately scored which brings the maximum score up to 8 This method has been applied by the author since 1965 in his daily clinical practice which proved that it is appropriate to simplify the method as described above no loss being incurred in the power of discriminating shunts with less than 50 % of pulmonary flow from larger ones

The judging of subtle signs of chronic pulmonary oedema is a rather inaccurate process, which is susceptible to considerable variation of the results in repeated examinations as regards the grade of changes observed, whereas its reproducibility is better in respect of presence or absence of changes

FINDINGS

Heart Volume

The relative heart volumes before and after surgery were plotted against each other Fig 9 The heart volume was preoperatively normal in 24 patients (19 %) 20 of the young age group and four belonging to the older group (20 % and 14 % of the respective groups) Women presented normal heart volume slightly more frequently (in 20 %) than men (in 13 %)

Postoperatively normal heart volume was established in 66 patients (51 %) namely in 58 of those younger than 40 years and in eight older patients (57 % and 29 % respectively) Normal heart volume was then about equally common in women as in men (50 % and 47 % respectively) Of the three patients with residual shunt two had previously displayed normal relative heart volume and their postoperative value was even smaller the values noted in the third case before and after operation were 924 and 976 ml/m² BSA respectively The preoperative heart volume was larger than 1000 ml/m² BSA in four patients all of them aged 40 years or older and larger than 800 ml/m² BSA in 18 patients (14 %) The largest and smallest values recorded preoperatively were 1308 and 278 ml/m² BSA Postoperatively the largest heart volume recorded was 754 ml/m² BSA excepting the case with residual shunt mentioned above The heart volume remained unchanged postoperatively in 11 cases in addition to this case with residual shunt All these patients had had normal or almost normal heart volume preoperatively

The average heart volumes before operation in ml/m² BSA in the entire series (the cases with residual shunt excluded) in the group of patients younger than 40 years and in the old age group were 595 (S.D 121) 552 (S D 121) and 747 (S D 236) respectively The corresponding postoperative means were 463 (S D 96) 440 (S D 80) and 546 (S D 104)

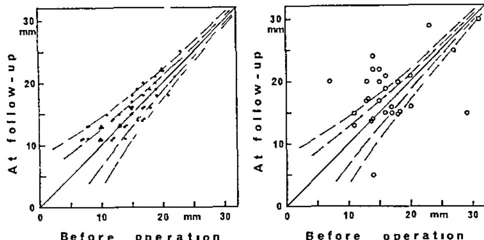


Fig 10 Amount of overlapping of the left ventricle and the inferior vena cava in the left lateral radiogram before operation and at follow up in 101 patients of the young age group (Fig 10 a) and 28 patients of the old age group (Fig 10 b) — On either side of the diagonal representing equal preoperative and postoperative overlapping the ± S D and ± 2 S D limits consistent with the findings in separate quintuplicate determinations (dotted lines) have been entered

The differences between the age groups as well as those between the preoperative and postoperative values in both groups are statistically highly significant (***)

Left ventricular — vena cava inferior overlapping

The amount by which the left ventricle and vena cava inferior overlapped was abnormally great before operation in altogether 15 subjects (those with residual shunt excluded) that is in 12 % of the entire series ten of these belonged to the group of patients younger than 40 years and five to the old age group (10 % and 17 % of the respective group) The number of patients presenting an abnormally high postoperative value was 20 (16 % of the series) 13 of them younger than 40 years and seven belonging to the old age group (13 % and 25 % of the group respectively)

In the entire series excluding residual shunts the overlap averaged 15 4 mm (S D 5 5) and 16 8 mm (S.D 4 6) before and after surgery respectively No statistically significant differences were established between groups nor as regards the change attendant on operation Fig 10 shows a diagram obtained by plotting the preoperative and postoperative values against each other It can be seen that in altogether 28 cases (22 %) the postoperative overlap surpassed the preoperative value by an amount exceeding the deviation of a single determination to be expected with 5 % probability on the basis of the replicate determinations by which the error of the method was assessed The number is made up of 19 cases belonging to the group of patients younger than 40 years and nine patients of the old age group (19 % and 32 % respectively) The overlap had decreaesed on operation by more than the expected deviation mentioned in 12 cases (9 % of the series)

Relative cross sectional area of the aortic arch

The cross-sectional areas of the aortic arch found before and after operation have been plotted against the patients age in Fig 11 which also contains the line of regression representing the relationship of the two variables in the normal control series and its 95 % toler ance limits Plots falling below this tolerance belt were preoperatively obtained in 25 cases (19 %) 13 of them belonging to the group of patients younger than 40 years and 12 to the old age group (10 % and 43 % of the respective group) The corresponding postoperative numbers are altogether 9 cases (7 %) two of them

a)

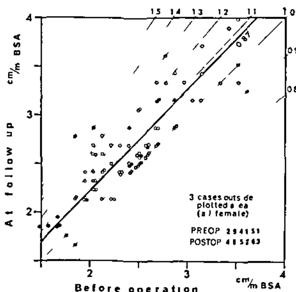
b)

Cross sectional area of aortic arch cm^2/m^2 BSA

Age, years

Fig 11 Correlation of the cross sectional area of the aortic arch relative to body surface area on age in 129 patients before (Fig 11 a) and after (Fig 11 b) operation (heavy regression line) — ● Male ○ Female patients The thin solid line represents the regression of the normal series (see text) with its 95 % tolerance limits indicated on both sides of it

Fig 12 Cross ectional area of the aortic arch relative to body surface area before operation and at follow up in 129 patients (heavy solid line linear regression) — ● Male ○ Female patients thin lines loci of constant ratio of postoperative and preoperative value (scale in the margin)



belonging to the young and seven to the old age group (2 % and 25 % respectively)

The cross-sectional area in cm^2/m^2 *BSA* averaged in the group of patients younger than 40 years and in the old age group 2 37 (S D 0 47) and 2 90 (S D 0 66) preoperatively and 2 58 (S D 0 33) and 3 22 (S D 0 96) postoperatively The difference between the means of the age groups was statistically highly significant (***) both before and after surgery

The cross-sectional area of the aortic arch relative to body surface area correlated fairly well with the age both preoperatively and postoperatively ($r = 0\ 52$ and $0\ 51$ respectively) the regression equations being $y = 1\ 67 + 0\ 28\ x$ and $y = 1\ 76 + 0\ 34\ x$ The regression lines are non parallel to that found in the normal series at a statistically highly significant level (***) before and at almost significant level (*) after surgery the slope of both is less steep than in the normal series Of the two lines the postoperative one has a slightly greater slope yet without any statistically significant difference The preoperative

and postoperative values which have been plotted against each other in Fig 12 are mutually correlated ($r = 0\ 87$) at a statistically highly significant level (***) and the difference between the means before and after surgery is statistically highly significant (***) Denoting the preoperative and postoperative cross-sectional area with y_1 and y_2, respectively the regression line has the equation $y_2 = 1\ 03\ y_1 + 0\ 15$ the line is found to be very nearly coincident with the line consistent with $y_2\ y_1 = 1\ 1$ This implies that the average increase in relative cross sectional area was approximately 10 % independent of the absolute preoperative value while it is true that the increase was slightly less marked in older individuals

Heart shape pulmonary vessels and pulmonary interstitial oedema

Fig 13 in which also the cases with residual shunt have been included presents a compilation of the findings concerning heart shape pulmonary vasculature and interstitial oedema There was no case in which all radiological findings would have been normal

The *right ventricle* was radiologically enlarged before operation in nearly every case particularly in the patients aged 40 years or older of whom 64 % presented enlargement of grade 2 The right ventricle was postoperatively normal in size in about half of the series but in the group of patients aged 40 years or older this percentage was only 21 %

The *left ventricle* was judged to be enlarged about twice as often in the group of patients aged 40 years or older as in the young age group both before and after surgery Enlargement was slightly more common after than before the operation Left ventricle referred to the normal category but apparently of abnormally small size was noted in 21 % of the cases preoperatively and in 4 % postoperatively

The *right atrium* was enlarged in approximately 70 % of all cases before surgery changes of grade 2 being commoner by a factor

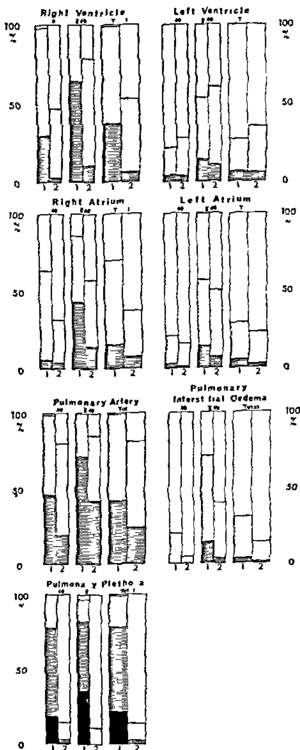


Fig 13 Frequencies in percent of various radiological findings concerning heart shape and pulmonary vasculature before (1) and after (2) operation — Blank Normal finding Singly hatched Change from normal of Grade I Cross hatched Change of Grade II

of 7 in the old than in the young group After the operation enlarged right atrium was still observed in 38 %

The *left atrium* was found to be enlarged preoperatively in only 21 % of the young age group but in 57 % of the old age group one-quarter of which presented enlargement of grade 2 The postoperative findings were closely equivalent, yet with a slight change towards normal

The *pulmonary artery* was preoperatively enlarged in nearly all cases and still in 81 % postoperatively although then the degree of enlargement was less marked Here too greater changes attendant on operation were displayed by the old than by the young age group

Pulmonary plethora was evident in nearly all cases preoperatively However in one female patient aged 55 years with large ($Q_p/Q_s = 2\,3$) left-to right shunt only inconclusive signs of the shunt were displayed by the pulmonary vasculature Pulmonary vascular markings of grade 3 were preoperatively seen in 21 % and postoperatively in none of the cases Abnormal pulmonary markings suggesting plethora were still present after surgery in 13 % although there were only three patients (2 3 %) with residual left-to-right shunt Fluoroscopy revealed hilar dance in 94 % of the cases preoperatively but in only six cases postoperatively three of which had a residual shunt

Anomalous pulmonary venous drainage was present in eight cases It was detected by tomography in all but one of the cases involved False positive diagnosis occurred in two cases In one case with postoperative residual shunt no anomalous pulmonary vein was found by preoperative tomography nor was any detected at operation although the preoperative oxymetric data were highly suggestive of such anomaly

Pulmonary interstitial oedema was preoperatively observed in 31 % of all cases also changes of grade 2 were present in the group of patients aged 40 years or older in 14 % The prevalence of positive findings after gery was only half of that before operation

COMMENTS ON RADIOLOGICAL FINDINGS

There was no case with completely normal radiological findings before surgery, and postoperatively there were eight such cases all of them patients younger than 40 years

The preoperative values of the heart volume were compared to the respective evaluations of arteriovenous oxygen difference *(AVD)* pulmonary-to-systemic flow ratio (Q_p/Q_s) right ventricular peak systolic pressure (P_{RV_s}), the $Q_p/Q_s \times P_{RV}$ product and the right ventricular stroke volume index (SVI_{RV}) No correlations were established except for a weak negative correlation between *AVD* and heart volume The correlation established by Jonsson et al (1957) and by Davidsen (1960) between heart volume and magnitude of the left-to-right shunt in ASD could not be elicited in the present series nor in any of its subgroups This conforms to the observations of Arnfred (1967) who found very wide scattering and poor correlation when he plotted the heart volume against pulmonary flow rate In adults this lack of correlation may be partly related to secondary myocardial changes in particular to disproportionate enlargement of the right atrium In three of the present cases with obstructive pulmonary hypertension the relative heart volume was slightly larger than in 13 cases with hyperkinetic pulmonary hypertension or incipient pulmonary vascular disease (averaging 879 and 768 ml/m² *BSA* respectively) somewhat at variance with the findings of Besterman (1961) This is probably accountable to the fact that the left to-right shunt was still considerable in magnitude in these cases

The relative heart volume was distinctly greater in the group of patients aged 40 years or older and it was associated with greater disability, as also other authors have found (e g Campbell et al 1957 Davidsen 1960 Arnfred 1967 b) However also normal heart volumes were found in the old age group and the prevalence of normal heart volume in the present series of adult patients was in the same order as in those of Reindell et al (1962) Sellers et al (1966) and Petersson (1967)

Reduction of the heart volume was a rather constant postoperative finding its average amount being 20 % in the patients younger

than 40 years and 27 % in the old group referred to the preoperative values Also Arnfred (1967 b) noted postoperative reduction of heart volume by 20 % In fact the changes in heart volume after surgery were larger in the old age group which differs from the observations of Loogen et al (1961) who failed to find any postoperative reduction in 30 % of their cases in addition to which such reduction was even less frequent in older patients In the present series postoperative reduction of heart volume only failed to occur in one case with large left-to right residual shunt and with pulmonary hypertension if the cases with less than 500 ml/m² BSA preoperative heart volume are disregarded Persistent abnormally large heart volume is thus of value in discriminating cases with residual shunt from those in which surgery has produced a favourable result even though the other two patients with a very small residual shunt in the present series displayed reduction of the heart volume by 15 % and 18 % respectively

The abnormalities found in the chambers of the right heart and in the pulmonary artery approximate those reported by most previous authors and cited in Chapter II They did not correlate on the pressure or flow parameters except for the typical peripheral pruning of pulmonary artery branches in pulmonary hypertension Tomography proved a highly useful and reliable means of detecting anomalous pulmonary veins Preoperative fluoroscopy revealed hilar dance in all cases including those with pulmonary hypertension (cf Schrire et al 1963) whereas this observation was only made in six cases postoperatively including all three cases with residual shunt of whom one presented an entirely normal radiogram Accordingly fluoroscopic examination for hilar dance possesses considerable value in respect of discriminating cases with residual shunt

The assessments of the magnitude of the shunt made from the peripheral pulmonary vascular markings were compared with the haemodynamic findings There was no correlation between $Q_r/\dot{Q}_s$ and vascularity in the present series nor was the result different in any way if the original scoring up to 8 points was applied This is probably associated with the fact that the left to right shunt was larger than 50 % of pulmonary flow in nearly all patients of the present series and to the circumstance pointed out by Fouche et al (1963) that the method is most efficient in distinguishing cases with a 50 % shunt from those with smaller shunts

Signs of pulmonary interstitial oedema were rather uncommon According to the literature septal lines are only rarely encountered in ASD (Rossall and Gunning 1956 Steiner 1959) Septal lines are occasionally seen when the left atrial pressure is between 15 and 24 mm Hg and invariably if it is higher than 24 mm Hg (Rossall and Gunning 1956) Uhley et al (1962) demonstrated by dog experiments that although acute pulmonary oedema due to artificial elevation of the left atrial pressure up to 40 mm Hg caused increase in pulmonary lymph flow up to twice or three times the control level chronic elevation of left atrial pressure to 10 mm Hg increased the lymph flow to a level higher by a factor of ten and pressure of 15 mm Hg by a factor of 25 When oedema fluid accumulates in the perivascular tissues of the pulmonary vessels which are rich in lymphatics the vessels involved acquire a hazy and indistinct outline (Logue et al 1963) Thus the physiological basis for occurrence of subtle signs of pulmonary interstitial oedema may exist in cases with moderately but chronically elevated atrial pressure particularly if the pressure is elevated in systemic veins into which the lymphatics drain The mean left atrial pressure was measured preoperatively in 29 cases displaying radiological signs of pulmonary interstitial oedema of grade 1 or 2 and in 57 cases without such signs The pressures averaged 7 18 mm Hg (S D 2 87) and 5 17 mm Hg (S D 2 75) respectively the difference being statistically significant (**)

Left atrial enlargement was not uncommon in the group of patients younger than 40 years and it was quite common among those of the old age group According to some authors the left atrium is not enlarged in ASD (Derra et al 1965 Spitz 1967) while in some other series it has been found to be enlarged in 9—55 % of the cases (Bedford et al 1941 Sommer and Voudoukis 1961 Novack et al 1963 Sellers et al 1966) particularly in old subjects Some authors attribute its occurrence to mitral valvular disease (e g Schrire et al 1963) It is possible that preoperative judging of the left atrial size is susceptible to

misinterpretation owing to backward rotation of the heart (Spitz 1967) In the present series however left atrial enlargement observed preoperatively was little influenced by the operation which could be expected to reverse the rotation if there was any

In many cases the left ventricle was visually found to be slightly enlarged after surgery compared to the preoperative finding and it was distinctly larger on the average in the group of older patients than in the young age group In the literature enlargement of the left ventricle is only mentioned by Novack et al (1963) and by Sellers et al (1966) It was found by the latter authors in 29 % of patients over 40 years of age and by the former in 13 % of 275 cases mainly in older subjects A similar result was obtained in the present study when the left ventricular-vena cava inferior overlapping was assessed which probably rather constitutes an underestimation as has been mentioned

The cross-sectional area of the aortic arch presented on the average abnormally small size before surgery in the present series This feature is a characteristic of ASD known since old (Bedford et al 1941 Nerard 1948 Saltzman 1954 Campbell et al 1957 Davidsen 1960 etc) Nerard considered the small aortic arch to be some kind of corollary of the large pulmonary artery Schrire et al (1963) attributed it to abnormal projection resulting from backward rotation of the heart or to true hypoplasia Jonsson et al (1957) Storstein and Efskind (1963) and Tikoff et al (1965) regarded the small aorta as a sign of small systemic flow The existence of abnormally small aorta in ASD has recently been disputed by some authors (e g Arvidsson 1963 Spitz 1967) who consider its small appearance only relative and not true and attribute it to misinterpretation induced by the large size of the pulmonary artery Arvidsson s (1963) angiographic observations on 10 children with ASD undoubtedly furnish no support for the concept of small aorta in ASD in children In the present series of adults however good evidence was gained of abnormally small cross-sectional area of the aortic arch in addition to which as a rule this area tended to increase by about 10 % on the average after surgery In the present series the average follow up time was 2.5 years The age-dependent increase of the cross-sectional area during a period of this length is only able to account for 1/5 to 1/3 of the increase observed It is not impossible that the small size of the aorta in ASD might be partly or completely related to hypokinetic circulation which has been shown to be rather characteristically associated with the condition in question e g by Petersson (1967) as well as in the present study (cf Chapter XI) The anomalies observed in aortic size were related to the flow and pressure parameters of left ventricle and aorta, but no correlation at rest or during exercise was established Nevertheless the size of the aorta and that of other arteries as well seems to be related to some extent to circumstances of flow and pressure as is borne out by abnormal size of the aorta found in arterial hypertension (e g Gustafson and Friedenberg 1965 Tibblin 1967) and by the relationships found between the intact and fistulated iliac arteries in connection with experimental iliac arteriovenous fistula (Koskinen et al 1967) Postoperatively slight but distinct changes seem to occur commonly in the left heart and aorta which are reciprocal to the marked reduction in size of the right heart and pulmonary artery and this is perhaps attributable to the general changes occurring in haemodynamics after closure of the defect that is abolishment of the shunt and change toward normal of the premises for left heart function

SYNOPSIS

The radiological findings of 129 patients with ASD secundum before and after surgical correction of their defect were analyzed in respect of heart volume relative to body surface area shape of the heart and pulmonary artery pulmonary vasculature signs of pulmonary interstitial oedema left ventricular-vena cava inferior overlapping and cross sectional area of the aortic arch relative to body surface area

The preoperative radiological findings were not completely normal in any instance while postoperatively normal findings were elicited in eight cases all of them patients younger than 40 years

The heart volume was normal before and after surgery in 19 % and 51 % of the cases respectively The highest preoperative value

was 1308 ml/m² *BSA* the limits of 800 and 1000 ml/m² *BSA* were surpassed in 18 and 4 cases respectively Eleven of the patients presenting large heart volume were 40 years or older Postoperatively the value of 800 ml/m² *BSA* was only exceeded in one single case involving a large residual shunt and pulmonary hypertension The reduction in heart volume attendant on operation averaged 20 % in the group of patients younger than 40 years and 27 % in the old age group

As regards the shape of the right heart chambers and of the pulmonary vessels the well-known typical abnormalities were preoperatively seen Right atrial and right ventricular enlargement were noted in 70 % and in 99 % respectively Abnormally large pulmonary artery and signs of pulmonary plethora were encountered in 99 % of the cases and subtle signs of pulmonary interstitial oedema in 21 % The postoperative incidences were enlarged right atrium 37 % enlarged right ventricle 53 % enlarged pulmonary artery 81 % enlarged pulmonary peripheral vasculature 14 % slight signs of interstitial oedema 15 % More or less distinct hilar dance was revealed by fluoroscopy in 94 % of the cases preoperatively and in six cases (5 %) postoperatively the latter including all three cases in which there was a residual shunt All findings were distinctly more pronounced in the old age group

On the left side of the heart the left atrium was preoperatively enlarged in 29 % and the left ventricle in 27 % of the cases The left ventricle seemed to be small in size in 21 % The corresponding postoperative percentages were 23 % 35 % and 4 % The cross-sectional area of the aortic arch related to age was significantly smaller than normal both preoperatively and postoperatively but it was larger (by about 10 % on the average) after than before surgery

The preoperative findings and the changes found in them after operation were compared with the main flow and pressure parameters of the right and left heart No correlations were established except for a weak negative correlation between the heart volume and arteriovenous oxygen difference and significantly higher left atrial mean pressure in cases presenting signs of pulmonary interstitial oedema than in cases without such signs However the slight but distinct changes observed postoperatively in the left heart and aorta which are reciprocal to the marked reduction in size of the right heart and pulmonary artery should be attributed to the general changes in haemodynamic conditions in the heart caused by surgical correction of the defect

IX SUBMAXIMAL BICYCLE EXERCISE TEST

METHODS

Equipment and procedure

In all the tests an electrically braked bicycle ergometer (Holmgren and Mattson 1954) was used which had been calibrated by the manufacturer (Elema-Schönander Sweden) prior to the tests In all but the tests conducted during right heart catheterization, which are considered in Chapter XI, the patient was in sitting position. The patients were instructed to pedal at constant rate 50—70 cycles per minute The method of Sjöstrand (Sjöstrand 1947 1967 Wahlund 1948) for estimation of physical working capacity at a given heart rate (150 or 170 beats/min) was applied in the submaximal exercise tests. The initial load was usually chosen to be 300 kpm/min for women and 400 kpm/min for men. It was increased by individually adjusted steps in accordance with heart rate response at intervals of 6 minutes, until the steady state heart rate approximated or exceeded the end point chosen in advance Three different loads were applied in nearly all of the tests. The ECG was continuously monitored and it was recorded, for the purpose of heart rate counting, every second minute with 50 mm sec paper speed Sjöstrand s steady state criteria in respect of heart rate change during the last four minutes of each period were applied in interpreting the records. The test was discontinued before the heart rate end point was reached if there ensued severe dyspnoea, exhaustion or chest or leg pain or in the event of any arrhythmia or cerebral circulatory disturbance The latest steady-state heart rate then determined the end point by which the working capacity was estimated

The working capacity corresponding to the given heart rate was found by interpolation or by extrapolation over a narrow interval. In the preoperative tests the end point of 150 beats per minute was specified, because many of the patients could not endure any longer runs or higher loads. The corresponding value was set at 170 beats per minute in the tests after surgery Throughout the study as constant conditions as possible were maintained in respect of time of testing food intake etc (cf Taylor et al. 1955)

Comments on methods and interpretation

For a check on the reproducibility of the bicycle ergometer test, 15 patients were subjected to duplicate determination on two consecutive days The coefficient of variation of the single determination was 9 6 % The same apparatus was used in all determinations The pedalling resistance has been found to change sometimes up to 30 % between calibrations when the load was less than 300 kpm/min but never more than 10 % at higher loads (Granath 1965) The same 15 patients were tested in some runs at low load on consecutive days, using other equivalent bicycle ergometers and the variation in steady heart rate was found to stay within the error of method stated above

It was not considered appropriate to estimate the working capacity by means of extrapolation over a wide interval from the heart rate end point obtained in the test Since many of the patients could not endure prior to surgery any loads higher than that producing a heart rate of 150 beats per minute this was chosen to be the end point in the preoperative tests. In the tests performed after the operation the increase up to 170 beats per minute was linear in nearly all instances PWC_{150} and PWC_{170} were both estimated by interpolation or narrow range extrapolation in these tests. The extrapolation needed to find the PWC_{170} value covered more than 10 beats per minute in six cases only

The control series comprised 70 apparently healthy persons of both sexes (25 women and 45 men) and of ages between 16 and 51 years, of whom a minority was trained in athletic exercise Most of the male subjects, of whom 44 originally belonged to a series presented by Frick (1962) had leptosome constitution. This series was augmented by a group of volunteers, mostly members of the hospital personnel. The PWC_{150} and PWC_{170} values found were plotted against body weight and against heart volume For PWC_{150} vs. heart volume and PWC_{170} vs heart volume the following regression equations were obtained with the independent variable x standing for heart volume and the dependent y for the working capacity $y = 0.598\,x + 311$ and $y = 0.734\,x + 409$ The regression equations with the heart volume (y) as independent variable are $x = 0.363\,y + 402$ for PWC_{150} and $x = 0.446\,y + 242$ for PWC_{170} The latter does not differ essentially in its slope from the control series of Holmgren et al. (1957) which was similarly mixed and consisted of subjects of both sexes whereas the volumes are lower in level on the average having been determined in upright position as opposed to supine position in the cited authors' study The working capacity is dependent on body weight, though in slight degree only according to determinations with the bicycle ergometer both in sitting and in recumbent position

(Wahlund 1948 Hertle et al. 1967 Sjöstrand 1967) The regression equations found in the present study for PWC_1 and PWC_{17} vs body weight, with x denoting the independent variable (body weight) and y the dependent variable (working capacity) are $y = 6.86x + 243$ and $y = 7.37x + 387$ These regression lines closely approximate the slope reported by Wahlund who examined 459 male subjects. In Fig 14b the regression lines specified above together with their 95 % tolerance limits have been entered The heart volume averaged 657 ml in the control series and the body surface area 1.76 m² Assuming that the subjects of the control series had a left to right shunt equal in average magnitude to that in the 37 preoperatively examined patients of the present series (i e $\dot{Q}_p/Q_s = 3.55$ on the average) their heart volume might be expected to be larger On the ground of Davidsen's formula (Davidsen 1960 $V = 200 + 100 \times PFR$) the expected mean relative heart volume would be 555 ml/m² BSA and the mean absolute heart volume 977 ml. This exceeds the actual average heart volume in the control series by about 320 ml Displacement of the lower 95 % tolerance limit in Fig 14b to the right (towards larger volumes) in the amount equivalent to 300 ml yields a coarse arbitrary estimate for a limit in which the expected increment of heart volume due to the left to right shunt has been taken into account and which is otherwise consistent with normal physical performance Changes not exceeding 10 % noted on comparison of the preoperative and follow up values were considered to be without significance The preoperative PWC_{150} values were originally reported in the series of Frick et al. (1966)

FINDINGS

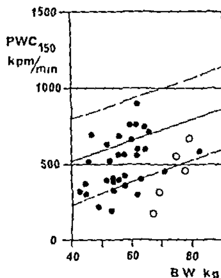
PWC_{150} was preoperatively determined in 37 cases 32 of them belonging to the group of patients younger than 40 years and five to the old age group (Fig 14a b) The postoperative determinations of PWC_1 and of PWC_{150} concern 123 patients At the follow-up tests one man of 41 years age discontinued the pedalling after 7 minutes on account of anginous chest pain During its presence typical ischaemic S T depressions were noted in his ECG One female patient of 36 years interrupted the test after 6 minutes at 300 kpm/min complaining of weariness of the legs and of fatigue No obvious cause for the fatigue was elicited other than exceedingly untrained condition normal performance was obtained after several weeks rehabilitation but this patient was excluded from the PWC determination series In a female patient aged 22 years 2 1 heart block developed at the heart rate of 142 per minute subsiding after the rate had gone down below 140 In another female patient of 18 years age who had complete A-V block there ensued 2 1 block during exercise One further test was discarded on account of technical fault

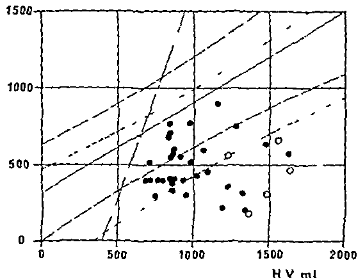
Work capacity at heart rate 150 per minute (PWC_{150}) before surgery and at follow-up in 37 patients

Plotting the preoperative PWC_{150} values against body weight (Fig 14a) the majority of the plots are seen to lie within the normal range although close to its lower (95 % tolerance) limit Plots falling below the 95 % tolerance belt of the normal regression are obtained in five cases (13 % of the series) two of them being patients younger than 40 years and three belonging to the old age group (6 % and 60 % of the group respectively) These five cases include the two cases of obstructive pulmonary hypertension occurring in the series and two out of four cases with atrial fibrillation

The plots of PWC_{150} over the heart volume (Fig 14b) display abnormally low performance in 26 cases (70 %) of which 21 belong to the younger and five to the older group (66 % and 100 % respectively) But if the normal regression is modified according to the hypothetical consideration of the effect from the left-to-right shunt there are only 11 plots (30 %) falling below normal range consisting of seven younger and four older patients (21 % and 80 % of the respective age group) In seven of these the $Q_p/\dot{Q}_s$ ratio was lower than the average found in the preoperatively examined series (3.55) it averaged only 2.7 in the 11 cases with low PWC_{150} in relation to heart volume These 11 cases include all four cases with atrial fibrillation the two cases with obstructive pulmonary hypertension and one of two cases with incipient pulmonary vascular disease Accordingly cases in which complications were present account for 64 % of the instances in which PWC_{150} was low in view of the heart volume

Significant increase in PWC_{150} attendant on operation is seen in altogether 26 cases (70 %) at follow-up of whom 22 belong to the young and four to the old age group (69 % and 80 % of the respective group) Eight patients all of them younger than 40 years presented no change while slight decrease was seen in two patients of the young age group and in one female patient of the older group who had atrial fibrillation





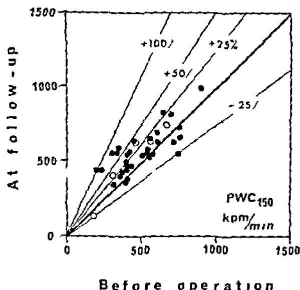


Fig 14 PWC_{150} values of 37 patients recorded before the operation plotted over their body weigh (Fig 14 a) and heart volume (Fig 14 b) and value at follow up plotted over the preoperative value (Fig 14 c) — ● Patients of the young age group o Patients of the old age group In Figs 14 a and b the solid lines and the inter rupted lines indicate the regression in the normal series with its 95 % tolerance limits the displaced closely dot ted lines stand for the hypothetical 95 % tolerance limits in pre ence of left to right shunt with $Q_p/Q_s = 3.55$ assuming normal PWC_{150} (see text) The steeply ascending line represents the normal regression of heart volume on PWC_{150} In Fig 14 c the inclined lines are loci of constant percentile change in PWC_{150} as a result of surgery

PWC_{150} averaged preoperatively 473 kpm/min (SD 28) in the entire series of 37 patients 479 kpm/min (SD 33) in the group of patients younger than 40 years and 436 kpm/min (SD 86) in the older group The corresponding means at follow-up were 585 kpm/min (SD 41) 596 kpm/min (SD 43) and 514 kpm/min (SD 109) The preoperative and postoperative means differ significantly (*) in the total series and in the younger age group

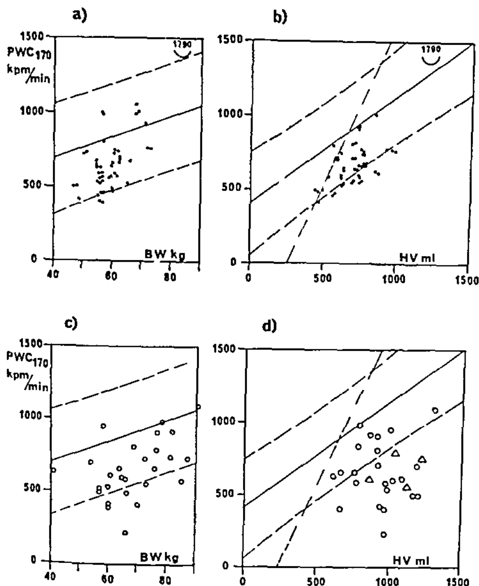


Fig 15 PWC_I values of 123 patients recorded after the operation plotted over their body weight (Figs 15 a and c) and heart volume (Figs 15 b and d) — ● Patients of the young age group o Patients of the old age group △ Patients with atrial fibrillation In the figures the corresponding regression in the normal series (see text) and its 95 % tolerance limits have been entered as well as the normal regression of heart volume on PWC_I

Work capacity at heart rate 170 per minute (PWC$_I$) at follow up in 123 patients

The PWC_{170} values plotted against body weight in Figs 15a c reveal that PWC_{170} was abnormally low in nine patients only (7 % of the series) Two of these belonged to the group of patients younger than 40 years and seven to the old age group (2 % and 26 % of the respective group) In seven of these nine cases (in 78 %) some complicating disorder was present namely incipient pulmonary vascular disease in two cases (out of altogether 10 in the series) obstructive pulmonary hypertension in one (out of three) atrial fibrillation in two (out of five) and residual shunt in one (out of three)

The plots of PWC_{170} against the heart volume (Figs 15b d) indicate that only 73 cases (59 % of the series) are within the 95 % tolerance limits of the normal regression in the rest the

value is lower than could be expected on the strength of the heart volume The 50 cases with abnormally low PWC_{m}, in relation to their heart volume comprise 33 patients younger than 40 years and 17 of the old age group (34 % and 63 % of the respective age group) 16 patients among those with low PWC_{m}, compared to heart volume (32 % of them) had some complicating disorder which was incipient pulmonary vascular disease in five cases (out of altogether 10 such cases) obstructive pulmonary hypertension in three (all cases of this kind), atrial fibrillation in five (all cases) and residual shunt in two (out of three)

The PWC_{1}, values at follow-up averaged 703 kpm/min (S.D 218) in the entire series 715 kpm/min (S.D 221) in the group of patients younger than 40 years and 660 kpm/min (S D 201) in the old age group No statistically significant difference exists between the groups The average PWC_{1}, in the control group was 657 kpm/min (S D 153) and the mean PWC_{m} in the entire follow-up series may thus be considered normal

COMMENTS ON THE EXERCISE TESTS

The work capacity in relation to body weight was abnormally low before surgery in a rather low proportion of the individuals examined In contrast when the values were related to the heart volume about two-thirds of the cases presented low work capacity Considering the heart volume-increasing effect of the left-to-right shunt consistent with 3 55 Q_p/Q ratio one-third of the values were still found to be low In these cases the heart *volume was not* proportional to the magnitude of the shunt on the arbitrary basis of judgement by Davidsen s formula (1960) stating the relationship of heart volume and shunt magnitude This is particularly emphasized by the respective cases Q_p/Q_s ratio of only 2 7 and it may be attributable to complicating myocardial changes Such changes may in part be irreversible and there may also be such changes in a number of cases with enlargement of the heart proportional to shunt magnitude in the sense stated above The findings made at follow-up support this conclusion in that PWC_{m} was then normal in relation to body weight in 93 % of the cases but only in 59 % of them when considered in relation to heart volume although the shunt was then absent

A wide range of submaximal work capacity and occasional very low values have been reported by Jonsson et al (1957) in ASD particularly related to heart volume but also to total haemoglobin which provides a far better basis for estimation of the predicted heart volume than does the body weight (e g Sjostrand 1967) Bohlau et al (1960) Schmutzler *et al (1960)* and *Reindell et al (1962)* have presented similar results of submaximal exercise tests and Knipping and Bolt (1959) of maximal oxygen uptake studies

Low work capacity was more common in the patients aged 40 years or older than in the younger age group both before and after surgery, as Jonsson et al (1957) have also pointed out On the other hand however improved work capacity after the operation was not less frequent in the old than in the young age group amounting to an approximately equal percentage Consistent improvement of work capacity after the operation was noted e g by Reindell et al (1962) They report that the change took place slowly in the course of several postoperative months Petersson (1967) observed no changes in work capacity after surgery in his series of relatively uncomplicated cases of ASD secundum

Preoperatively impaired work capacity was associated in two-thirds of the respective cases with complicating disorders such as atrial fibrillation incipient pulmonary vascular disease and obstructive or hyperkinetic pulmonary hypertension This is in good agreement with the findings made by Frick et al (1966) in their series concerning the work capacity in 96 cases of various congenital defects including 48 cases of ASD secundum These authors call attention to the possibility that in cases without any pulmonary vascular disease or left heart failure low work capacity may be due to poor training often as a result of caution induced by awareness of the heart defect The importance of obstructive disease of the pulmonary vascular tree as determinant of the working capacity in cases with left-to-right shunt has been analyzed by several authors with similar results (e g Swan et al 1958 Hugenholtz and Nadas 1963) In the present series the proportion of patients with low work capacity presenting complicating dis-

Table 12 Functional capacity before and after operation for ASD rated according to the criteria of N Y H A and by bicycle ergometer test (PWC_{1s}) in kpm/min

Functional Class (N Y H A)	Before operation N	Median	Range	At follow-up N	Median	Range
FC I	10	615	400—900	124	600	140—1470
FC II	20	415	200—710	14	520	270—790
FC III	6	375	320—560	4	440	300—525
FC IV	1	315	—	—	—	—

orders was the same preoperatively and at follow-up One of the present patients male illustrates the importance of atrial fibrillation as regards work capacity His atrial fibrillation was converted to sinus rhythm in the course of the follow-up examinations and his PWC_{1} was determined on consecutive days before and after this conversion After conversion the work capacity at the same heart rate was better than before by 23 % Such effect was already observed by Bruce and Rogers (1953) in their study on mitral patients However improvement of the work capacity is a fairly uncommon phenomenon after conversion if the heart rate is kept constant by means of pacing (Graettinger et al 1963)

The functional class according to N Y H.A correlated fairly well with the PWC_{1s} value (Table 12) although wide scattering could be noted Comparison of the preoperative and postoperative data suggests that the patients tended slightly to overrate their preoperative functional capacity compared to that after surgery This is contrary to the commonly stated contention that most patients underrate their preoperative condition and overrate it after the operation (e g Bruce et al 1965 Petersson 1967) This phenomenon is important to be aware of in the evaluation of medical history data and it may be partly attributable to the method by which the history was taken in the present study

SYNOPSIS

The submaximal work capacity at the heart rate of 150 per minute (PWC_{1s0}) was determined by means of the bicycle ergometer with the patient in sitting position in altogether 37 cases of ASD prior to surgery and at the heart rates of 150 and 170 per minute (PWC_{1}) in 123 cases at follow-up after the operation

PWC_{1s} related to body weight was preoperatively abnormally low in five cases (13 % of the series) and in relation to heart volume in 11 cases (30 %) if the effect of the shunt on heart volume was taken into account and in 26 cases (70 %) if its presence was disregarded Low values occurred more commonly in the group of patients aged 40 years or older than in the young age group Significantly increased PWC_{1s} (by 10 % or more) was presented at follow-up by 26 patients (70 %) equally often in both age groups

PWC_{1} related to body weight was postoperatively abnormally low in nine cases (7 % of the series) and in relation to heart volume in 50 cases (41 %) indicating the likelihood of irreversible myocardial changes PWC_{1} was normal on the average in the entire series and in its both age groups However low values were commoner in the old age group

The group of patients presenting abnormally low submaximal work capacity before and/or after the operation included most of the cases with complicating disorders such as incipient pulmonary vascular disease hyperkinetic or obstructive pulmonary hypertension atrial fibrillation and residual shunt at follow-up

The classification by functional capacity according to the N Y.H.A criteria was found to be in fair agreement on the average with the results obtained in the exercise tests although wide scattering accompanied the correlation Comparison of the correlations established in preoperative evaluation and at follow-up revealed that the patients tended slightly to overrate their preoperative condition as compared to rating of the postoperative condition

X LUNG FUNCTION TESTS

METHODS

The lung function tests were all performed as routine determinations in the hospital s laboratory In all postoperative tests and in part of those carried out before surgery a Bernstein spirometer (Kifa, Sweden) was used and in the rest of the preoperative test other types of spirometers (Broncho spirometer Lundia Sweden Pulmometer Godard Holland) An analysis was made in the present study as regards lung capacities only of the vital capacity (*VC*) and in respect of ventilatory functions of the one second forced expiratory volume ($FEV_{1.0}$) For correction to *BTPS* the measured values were multiplied by the factor 1 10 regardless of the room temperature and barometric pressure in each particular instance The final analysis concerns only values of *VC* in percent of predicted value and those of FEV_{1} in percent of actual *VC* The expected values are based on experimental data obtained with normal subjects, which have been reported by Poppius (1965) In the majority of the preoperative volume determinations the sitting and recumbent position both were considered postoperatively the sitting position only *VC* is normally 7 0—7 7 % lower in recumbent than in sitting position (Svanberg 1957) The difference noted in the present series was only 1 0 % of the predicted *VC* (S D 6 9 %) In another series involving ASD (not included in the present series) double determinations of the reduction in *VC* attendant on transition from sitting to recumbent position were made in 12 cases which revealed that the value obtained in recumbent position was smaller by 4 0 % (S D 2 6 %) *Approximately simi* lar observations of small influence of the posture on the transfer factor for carbon monoxide in ASD have been made by Tala et al (1968) In the *VC* determinations the sitting and recumbent posture were employed in 53 and 114 cases respectively The *VC* measured in recumbent position was com pared to the postoperative values. Taking into account the influence exerted by posture and the standard error of one single determination, which was 2 7—3 6 percent of the spirometric value in respect of *VC* and 3 6—3 8 in respect of $FEV_{1.0}$ (Poppius 1965) the limits to be interpreted as a significant change from preoperative to postoperative value was fixed at the round figure ± 15 % of predicted value for *VC* and at ± 10 % of actual *VC* for FEV_{1} Both *VC* and $FEV_{1.0}$ values less than 80 % were considered to be abnormally low

FINDINGS

Vital capacity

The preoperative and postoperative *VC* values were both available in 118 cases (91 young and 27 old patients) including the three cases with residual shunt

The preoperative *VC* was normal in 52 patients younger than 40 years and in 12 of the old age group (57 % and 44 % of the respective age group) or in altogether 64 cases (54 %) The other patients presented reduced *VC* which was less than 60 % of predicted value in six of the younger and three of the older patients The corresponding postoperative numbers were 71 and 16 (78 % and 59 %) in the two age groups and 87 (74 %) in the entire series and less than 60 % of predicted value was recorded in one patient younger than 40 years only Significantly increased *VC* was noted at follow-up in 22 of the younger and eight of the older patients (24 % and 30 % respectively) or in 30 (25 %) of all patients examined *VC* had been significantly reduced at follow-up in three patients younger than 40 years

VC averaged preoperatively 81 2 % of predicted value (S D 13 8 %) in the entire series and 82 3 % (S.D 13 8) and 77 6 % (S D 13 3) in the young and old age group respectively The corresponding averages at follow-up were 88 8 % (S.D 12 9) 89 2 % (S D 12 7) and 87 3 % (S D 13 7) A statistically significant difference was established between the preoperative and postoperative values in the entire series (***) in the young age group (***) and in the old age group (*)

In two of the three cases with residual shunt no change in *VC* was noted postoperatively in the third case in which pulmonary hypertension was present there was a slight yet not significant reduction

Forced expiratory volume

The values of FEV_1, determined both before and after surgery were available in 50 cases 37 of them being patients younger than 40 years and 13 being older patients No cases with residual shunt were among these

FEV_1, was preoperatively normal in 29 cases (58 % of the series), namely in 25 of the younger and four of the older patients (68 % and 31 % of the respective group) The corresponding postoperative figures are 35 (70 %) and 31 and 4 (84 % and 31 %) in the two age groups All the other values were lower than normal less than 60 % was recorded preoperatively in two patients younger than 40 years but po toperatively in none Significantly increased FEV_1 was observed at follow up in eight cases (16 %) consisting of six younger and two older patients (16 % and 15 % respectively) One patient younger than 40 years displayed significant reduction of FEV_1

The preoperative FEV_1 averaged 78 1 % of actual VC (S.D 12 0) in the entire series and 80 9 % (S.D 12 8) and 74 0 % (S.D 11 8) in the young and old age group respectively The corresponding averages at follow-up were 83 7 % (S D 9 2) 86 6 % (S D 8 5) and 74 0 % (S D 9 0) The difference between the preoperative and postoperative means in the entire series was significant (*) but not in either of its age groups

One of the cases with residual shunt presented a postoperative FEV_1, unchanged from its preoperative value while slight reduction had occurred in the other two

COMMENTS ON LUNG FUNCTION TESTS

The VC and FEV_1 were both lowered from normal before surgery in nearly half of the cases examined more commonly in patients aged 40 years or older The postoperative values were higher on the average differing significantly from those found before operation Improvement of VC and of FEV_1, was more frequent in the group of patients younger than 40 years than in the old age group

Of the cases displaying improvement of VC 30 had preoperatively significantly (**) larger heart volume and presented postoperatively slightly greater reduction in heart volume than those in the rest of the series (27 % and 20 % respectively) The radiological assessment of pulmonary plethora before surgery revealed vascular markings of grade 3 in 33 % of the cases which showed postoperative improvement of VC contrasted with 20 % in the rest of the series Interstitial oedema of grade 1 was radiologically observed in 40 % of the first-mentioned and in 25 % of the last mentioned cases

The $Q_P/\dot{Q}_s$ ratio and the peak systolic pressure in the pulmonary artery were not different on the average in these two group Significant improvement of VC noted at follow-up was thus often associated with more conspicuous changes in heart volume and radiological findings as regards pulmonary vasculature while it did not seem to bear any relation as a rule to the flow and pressure parameters of lung circulation It is to be noted though that the two cases with hyperkinetic pulmonary hypertension both displayed reduced VC preoperatively as well as after surgery The VC value was low in two out of three cases with obstructive pulmonary hypertension preoperatively and in all three postoperatively Of 13 cases with incipient pulmonary vascular disease five presented reduced VC before the operation but only two at follow-up one of the latter having a large residual shunt It is thus seen that pulmonary vascular disease and/or pulmonary hypertension was perhaps slightly more often associated with reduced VC than was the case in absence of such complications but there were many exceptions to this trend Consistent with this finding is that reported by Woolf (1963) In 36 cases reported by Petersson (1967) VC was normal both before and after surgery which may be partly accountable to the absence of complications in his series In the nine cases of ASD secundum of Strano et al (1967) VC was lower than the excepted value by 8—9 % — The postoperative change in VC takes place slowly after surgery In nine cases of ASD (not included in the present series) we still elicited slightly lower VC values than preoperatively when the measurement was made 9—16 weeks after the operation (Tala et al 1968)

In a considerable proportion of the patients with reduced preoperative VC signs of ventilatory insufficiency developed in the early postoperative period Also Ariza et al (1964) have stressed the importance of VC assessment as a single test in distinguishing patients dis-

posed to ventilatory failure after cardiac surgery

Airway obstruction manifesting in reduced FEV_1, was not related in any degree whatsoever with the flow and pressure parameters of the lung circulation and the radiological findings in cases with low FEV_1, were not dissimilar to those made when FEV_1, was normal Only one-third of the patients with preoperatively reduced FEV_1, had experienced nocturnal paroxysmal dyspnoea or orthopnoea or developed oedema of the leg Only one such patient had pulmonary hypertension Airway obstruction was a finding twice as frequent in the group of patients aged 40 years or older as in the young age group and even though it was not severe it did not improve much in the cases in which it occurred Granath and Strandell (1964) noted positive correlation of airway obstruction and the filling pressures of the ventricles during exercise in old men In the present series the filling pressures found during exercise in patients for whom the postoperative test yielded reduced FEV_1, values did not deviate from the general pattern seen in the groups

Unfavourable result of surgery was reflected in the present series by absence of improvement or deterioration of the results elicited in the lung function tests Results of this kind were rare in cases without pulmonary hypertension in which repair of the defect had been successful regardless of age

SYNOPSIS

Vital capacity (VC) and forced expiratory volume (FEV) were determined before and after surgical correction of ASD Only those cases of the material were subjected to analysis in which the preoperative and postoperative values were both available These cases number 118 as regards *VC* and 50 for FEV_1.

VC was normal in 54 % of the cases preoperatively and in 74 % postoperatively with higher frequency of normal values in the group of patients younger than 40 years than in the old age group Significant improvement of *VC* was noted at follow-up in 25 % of the cases about equally in both age groups A statistically significant difference existed between the means before and after surgery in the total series as well as in both age groups The impairment of *VC* whenever observed was only moderate in most instances Improvement was often associated with marked reduction of the radiological heart volume and of pulmonary vascular markings but not with changes in the flow and pressure parameters in the lung circulation In cases involving pulmonary hypertension improvement of *VC* was less common

FEV_1, was normal in 58 % of the cases preoperatively and in 70 % postoperatively the frequency of normal values in the group of patients aged 40 years or older was only half of that in the young age group However a statistically significant difference was established between the preoperative and postoperative means of the total series Reduced FEV_1, was not related with any haemodynamic or radiological findings nor with history of heart failure The frequency of such reduced values was only slightly affected by the operation

The cases with residual shunt displayed no improvement of *VC* or FEV_1, in the lung function tests performed after surgery but the same also was true in many cases presenting good results of surgery

XI HAEMODYNAMIC FINDINGS

The haemodynamic studies consisted of determination of pulmonary and systemic blood flow by oxymetric methods estimation of the size and site of potential shunt flows by oxymetry and by indicator dilation technique measurement of blood pressure in various parts of the central circulation and calculation of various haemodynamic parameters from the data thus obtained All the patients were catheterized before surgery and dye dilution tests were combined with this examination in 117 cases After surgery heart catheterization was performed on 58 patients tests by the indicator dilution technique were made in all cases either in connection with catheterization or when no recatheterization was performed by peripheral injection technique Both preoperatively and postoperatively the pressure-flow determinations were made in supine position at rest in all cases and after surgery also during supine exercise in 54 cases

METHODS

Determination of systemic pulmonary and shunt blood flow

The blood flow was determined according to Fick s principle The expiratory gases were collected in Douglas bags fitted with low resistance valves with 5 min sampling time at rest and 3 min during exercise Shortly after completed collection, the volume of the gas was determined with a gasometer and its composition was elicited partly with Scholander s apparatus and partly with the katharometer In the determinations of oxygen consumption, the standard deviation of one single determination was 9 5 ml (coefficient of variation, 4 3 %) No measurements of the basal oxygen consumption were made it was instead calculated from the predicted caloric value of the basal metabolism obtained from standard multiple prediction tables (Harris and Benedict 1918) for normal adult men and women. The average caloric value of 4825 kcal was assumed for oxygen in the calculations corresponding to RQ of 0 82, which is most commonly observed in fasting subjects (Houssay 1955) In respect of subjects younger than 21 years the table of Boothby et al (1938) was used which yields values higher by 2–5 % for young adults as compared with those of Harris and Benedict. The gas volumes have all been stated as volumes at *STPD*

The blood samples for determination of the blood oxygen soturation (S_{O_2}) and oxygen content (C_{O_2}) were drawn during a short time interval and whenever possible while the collection of respiratory gases was in progress. At the preoperative catheterizations superior caval blood was used instead of mixed venous blood in determining the systemic arteriovenous oxygen difference *(AVD)* Pulmonary arterial and pulmonary venous blood samples were used for the determinations of shunt flow magnitude If no pulmonary venous blood could be obtained, its oxygen saturation was assumed to be 95 % In the postoperative examinations, mixed venous blood samples were drawn from the pulmonary artery both at rest and during exercise

Analysis of the C_O and S_O was carried out in one quarter of the preoperative determinations by the direct manometric method of Van Slyke in addition to indirect spectrophotometric analysis by the standard method of Drabkin et al. (1949) and of Nahas (1951) The haemoglobin was photometrically determined The standard deviation of one single determination in the S_{O_2} analyses was 1 09 % (coefficient of variation, 1.5 %) and in the C_O analyses it was 0.19 vol. % (coefficient of variation 1.5 %) The difference between the absolute values of S_O found by the two methods ranged from 2 to 5 % the spectrophotometric method yielding higher values No significant difference was established in respect of *AVD* however Fick s formula $(Q = V_{O_2}/AVD)$ was used in calculating the systemic flow (Q) pulmonary flow (Q_p) and effective pulmonary flow (Q_e) the respective arteriovenous differences being substituted in this equation (i e $AVD = C_{aO_2} - C_{vO_2}$ $AVD = C_{PVO_2} - C_{PAO_2}$ and $AVD = C_{PVO_2} - C_{SVCO_2}$ *respectively)* The left to right shunt was calculated as the difference $Q - Q$ and the right to left shunt, as the difference $Q - Q_e$ The coefficient of variation in ten paired determinations of the cardiac output at rest, amounted to 8 8 %

Pressure recording

The instrument used for pressure recording was an electromanometer of the strain gauge type (Sanborn) in one-quarter of the preoperative catheterizations and a variable reactance electromanometer of the inductive gauge type (EMT 490 A, Elema Schonander) in most of the rest. The D C or carrier preamplified signals from

the transducers were recorded with the aid of a two channel or six-channel direct writing ink jet recorder (Elema Schonander) simultaneously with the ECG lead II In all the postoperative measurements the Sanborn Poly-Beam Recording System was used Its electromanometer consisted of a variable reactance transducer of differential transformer type (Sanborn 267 B) and the signal from this was passed through the carrier amplifier (Sanborn 350—1100 B) to a four-channel recorder fitted with rapid developer (Sanborn 568—100 A + Sanborn 563) The ECG lead II was used to supply a reference record here too Standard radiopaque Cournand catheters (No 8 in most instances 100 or 125 cm long) with adapters were directly connected to the transducers At the preoperative recordings of arterial pressure the puncturing needle was connected with the mano meter by Teflon or Dacron tubing (4F) 50 cm in length The zero reference point was 10 cm above the table surface

As a test of the dynamic response characteristics of the pressure recording system each record was initiated with a square wave impact For the purpose of analyzing the dynamic response of the entire system at various frequencies a hydraulic pressure generator was moreover constructed according to Vierhout and Vendrik (1961) which consisted of an oscillator unit, a power unit and a loudspeaker pressure chamber unit. Catheters of the same kind as were used in the actual examina tion of patients were filled with saline they were kept in a water bath at 37 C and connected to the pressure chamber The latter was induced to generate hydraulic sinusoidal oscillations at frequencies ranging from 1 to 100 Hz When a No 8 Cournand catheter of 125 cm length and an EMT 490 A transducer together with the corresponding preamplifier were used the undamped natural frequency was found to be 50 Hz, the response at resonance + 275 % and the response was found to be flat (within ± 5 %) up to 18 Hz, and to have a rather steep rollof at frequencies higher than that of the resonance peak. In the instance of the Sanborn system with the same catheter the undamped natural frequency was 35 Hz + 300 % response was noted at resonance and the response curve was flat (± 5 %) up to 10 Hz, and displayed a steep rolloff above the frequency of resonance peak The degree of damping in the pressure measurements during catheterization was usually between 0 7 and 0 8

An electrical calibration reference was employed as a rule and frequent comparisons with a hydrostatic pressure reference were performed. An appro priate amplification was chosen for each pressure level so as to obtain a linear record from the systolic waves. The mean pressures were mostly evaluated by means of electrical integration, but integration by planimetry was used in some cases The mean systolic pressures were obtained by plani metric integration.

Indicator dilution technique

For indicator various dye solutions (Evans blue® Coomassie blue® Cardiogreen) were used In all in stances a photoelectric cell (Cambridge earpiece) and a preamplifier and recorder unit (Cambridge Dye Dilution Recorder Mark I) served to record the pas sage of dye through the pinna of the transillumi-nated ear The standard deviation of a single determination of the $C_{P\downarrow 15T}/C_P$ ratio was 0 05 and that of the C_L/C_R ratio 0 09 No quantitative estimations of the blood flow by means of the dye dilution tech nique were made in the present study

Procedure in present investigations

The examinees were in postabsorptive fasting state under catheterization and had been premedicated with 100 mg Nembutal® or 50 mg Phenergan® about one hour previously and they were in supine position during examination. The electrocardiogram was monitored throughout its progress In the majority of the cases right heart catheterization was perform ed by route of the basilic vein or one of its tribu taries while in some cases the saphenous vein was chosen. A No 8 standard Cournand catheter without side holes was thus introduced into the caval veins and right atrium and passed through the atrial septal defect into the left atrium often into the left ventricle and into the pulmonary veins Multiple blood samples were drawn in each position mentioned the pressures were recorded in each chamber and during retraction of the catheter to the right atrium The catheter was then introduced into the right ventricle and further into the pulmonary artery trunk into both main branches of the pulmonary artery and finally into wedge position In the course of this process multiple blood sampling and pressure recording was performed While the tip of the catheter was in the pulmonary artery the collecting of expiratory gases and the arterial blood sampling and arterial pressure recording were carried out. In most of the preoperative examinations the femoral artery and in some of them the brachial artery was percutaneously punctured for arterial sampling Finally the dye dilution curves were recorded, to which purpose dye solution was injected into the right and left pulmonary artery branch pulmonary trunk, often into the pulmonary veins and right atrium, and into the inferior vena cava (into the superior vena cava when sinus venosus defect was present) In the course of retraction of the catheter from the pulmonary artery during the dye dilution study continuous records were made of the pressures in order to obtain an idea of potential pressure gradients in the canal of the right heart

In the catheterizations after surgery mainly the same principles were followed as in the examinations made at rest The radial artery was exposed and cannulated with a length of nylon tubing for arterial blood sampling and pressure recording On conclusion of the sampling at rest, the tip of the catheter was impacted in wedge position in the right basal lung field under x-ray control and after a check for appropriate wedge pressure curve the catheter was connected to a continuous slow drip of 5 % glucose under 50 mm Hg pressure with 50 mg heparin sulphate per 500 ml in the infusion solution An electri cally braked bicycle ergometer (Elema Schonander) was attached to the table and the patient s feet were raised and placed on the pedals. The pressures were once more measured at rest in this position. The pa-

tient was fitted with a mask for collecting the expiratory gases and instructed to commence pedalling Only one load (usually 300—500 kpm/min) was applied This load was chosen on the basis of the observations made in the exercise test in sitting position with the aim that the heart rate would not be likely to rise much higher than 140 beats per minute After warming up exercise of two minutes duration, the expiratory gases were collected over the next three minutes of exercise During the fifth minute of the exercise period the pressures in systemic artery wedge position pulmonary artery right ventricle and right atrium were consecutively recorded and the arterial blood and mixed venous blood were sampled

Of the patients not subjected to postoperative catheterization a dye dilution curve was recorded with peripheral intravenous injection under reactive hyperaemia induced for the purpose of rapid flushing of the dye into the central veins (Bender and Koch 1960 Bender 1964) After puncturing the basilic vein with a needle connected to a syringe containing indicator dye solution the brachial artery was occluded by inflating the sphygmomanometer cuff to a pressure level higher than systolic arterial pressure for a period of four minutes Upon deflating the cuff 2 ml of the dye solution were rapidly injected into the vein, the arm was elevated for a period of ten seconds and the dye curve was recorded as has been described *In the majority of the cases two or several curves were consecutively recorded*

Comments on interpretation of the flow data

Various methods of mixed venous blood sampling have been employed in ASD Superior *caval blood* has perhaps been most commonly used instead of mixed venous blood (e g Dexter 1956 Jonsson et al 1957 Scebat et al 1957 Storstein and Efskind 1963) Some authors preferred in the calculation of the *AVD* the mean of the C_{O_2} in the inferior and superior caval samples (e g Davidsen 1960 Swan et al 1958) or the mean of *one* superior caval and two inferior caval samples (e g. Swan et al 1954 Rowe et al. 1961 Davies and Gazetopoulos 1966) An analysis was made in the present series of the differences between blood oxygen contents in superior vena cava (C_{SVCO_2}) right atrium (C_{RAO}) and pulmonary artery (C_{PAO_2}) after surgery in the cases without residual shunt Good correlation ($r = 0.897$) was established between C_{SVCO_2} and C_{PAO_2} of which the latter was higher by 0 13 vol % on the average A change in C_O from superior vena cava to pulmonary artery by 0 52 vol % or more may be considered to be statistically significantly different from zero (**) The averages of C_{SVO} and C_{RAO} of which the latter was higher differed by 0 41 vol % At their comparison a change of 0 78 vol % or higher may be considered statiscally significant (**) The significant increments at 99 9 % confidence level were 0 87 and 1 09 vol % respectively Increase of the C_{O_2} *from* superior vena cava to right atrium by 1.5 2 0 vol % would thus seem an appropriate criterion for suspicion of atrial left to right shunt, as several authors have stated (e g Dexter et al 1947 Derra et al. 1965 Storstein and Efskind 1963) However in some instances shunted *saturated* blood may only be detected in the right ventricle or pulmonary artery (Weidman et al 1957) Our practice in interpretation of the laboratory findings is to suspect left to right shunt at atrial level in the event of consistent rise in C_O by 1 0 to 1.5 vol % from superior vena cava to right atrium and or to pulmonary artery and to consider such shunt probable if the increase exceeds 1.5 vol % Positive confirmation of the ASD diagnosis with the aid of the catheter is only furnished by passage of the catheter through the defect from the right into the left atrium (Brannon et al. 1945)

The small difference between C_{SVCO_2} and C_{PAO} would seem to justify the notion that C_{SVCO_2} is a good approximation of C_{VO} at rest in these patients This conforms to recent observations by Nielsen and Fabricius (1968) who also calculated the relative contributions of superior and *inferior* vena cava to the mixed venous blood *In this respect they* found that at rest the blood flow of inferior vena cava was 48 % of the total but during exercise (at 150—400 kpm/min) about 75 % of the total and then the C_{IVCO} was much lower than the C_{SVCO} The reasonable site for mixed venous blood sampling in postoperative catheter examinations under exercise is therefore the pulmonary artery A different sampling of mixed venous blood at rest and during exercise may account for a small but insignificant error in plotting volues of *AVD* and Q_s on V_{O_2} because of the slightly (0.13 vol %) lower C_O in superior vena cava as compared with that in the pulmonary artery

The standard deviation of a single determination of C_{PAO_2} was 0 2 vol. % *in the present series In some* instances when there is a large left to right shunt and when C_{PO} is high, an error of this magnitude may introduce an error exceeding 50 % in the determination of Q_p, and even in the presence of a moderate shunt the resulting error may be ± 5 to 10 % Estimation of Q_p in ASD by Fick s method is thus always rather inaccurate as *Dexter* et al (1947) already pointed out. *The reproducibility* of the cardiac output determination by Fick s method in our laboratory is 8 8 % at rest and it may be assumed to be even better in measurements during exercise (Donald et al 1954) Holmgren and Pernow (1960) found it to be 8.2 % at rest and 5 2 % during exercise both in supine position. According to Donald et al. (1955) a steady state ensues in most test subjects within the first minute of exercise Holmgren and Pernow (1960) observed 4 % increase of the cardiac output from the fifth to ninth minute in moderate supine exercise but they elicited no statistically significant changes in *AVD* or stroke volume after the fifth minute

Comments on interpretation of the pressure data

It has been found by Fourier analysis of the harmonic content of the different intracardiac and intra-

vascular or extravascular pressure pulse waves that the most essential information is usually contained in the first three to six harmonics of the fundamental frequency (Porje 1946 Hansen 1949) The importance of the fundamental frequency is much superior to that of the harmonics in most pulse waves with the exception of the atrial pulse where the amplitude of the fifth harmonic still amounts to 1/5 to 1/4 of the fundamental (Patel et al. 1965) It follows that the available information is more uniformly distributed among several harmonics in the case of the atrial pressure curve for this reason, in the atrial pressure curves which Patel et al. resynthetized from the first 5 harmonics the slopes of rapid atrial waves are still markedly distorted, compared to the orginal curve the slow waves being less distorted

In most of the currently employed pressure recording systems with external manometer and hydraulic pressure transmission the range of flat amplitude response extends up to 15—20 Hz (Fry et al. 1957) as it did for the manometers used in the present study Harmonics above this limit suffer more or less pronounced distortion (exaggeration of their amplitude) If the heart rate does not rise above 140 per minute systems of the kind mentioned are able to record the main atrial waves up to the fifth harmonic with satisfactory accuracy

The pulse wave is distorted, at transmission through the catheter by multiple reflections of its frequency components by unequal attenuation of the different components and by their different transmission times (Fry et al. 1957) Particular influence on the latter is exerted by the damping of the system in that low damping causes less phase distortion but, on the other hand permits greater amplitude distortion to take place Reasonable degree of both amplitude and phase distortion is usually achieved when the manometer catheter system is arranged to have a damping factor of 0 7 (Hansen 1949) At the indirect recording of the left atrial pulse from the wedge pressure (Lagerlof and Werko 1949) the vascular channel distal to the wedged catheter tip that is the small pulmonary artery pulmonary capillaries and pulmonary veins constitutes such an extension of the manometric system which possesses variable viscoelastic properties beyond control It has been shown in humans that the backward transmission of pressure pulse from the left atrium to the pulmonary artery which is occluded by the catheter is often very low and variable depending in particular on the variable compliance of the pulmonary veins and arterioles, which on the other hand is largely determined by their mean transmural pressure (Caro et al. 1967) As result, even though the mean pressures in the atrium and in wedge position usually correlate well (Lagerlof and Werko 1949 Dexter et al 1950 Luchsenger et al. 1967) the transmission of different harmonics and their different phase distortions are unpredictable Connolly et al (1953) suggest that expansion of the venous channel by intermittent flushing with saline using 200 mm Hg pressure in the flushing system would yield better wedge pressure curves. That flushing may elicit more constant transmission, has been substantiated also by the author's experience In the present study however no more detailed analysis of the atrial wave form from the direct or wedge record was considered to be justified at any heart rates higher than 100 per minute

Similar problems are involved in the estimation of central aortic pressures from peripheral arterial pulse tracings Kroeker and Wood (1955) compared the pressure pulses obtained from various arteries and found that at rest, the systolic pressure in the brachial, femoral and radial arteries was 109 112 and 110 % respectively of the central pressure the corresponding percentages during exercise were 111 113 and 101 The diastolic and mean pressures amounted to 88—97 % of the corresponding central pressures The distortions in all the arteries mentioned were in the same order of magnitude which was also observed in the present series.

All heart cavities were entered with the catheter in part of the present series only For the calculation of certain parameters of ventricular function in all cases the pressure phenomena in the ventricles were therefore estimated from the atrial, wedge and arterial pressure tracings in disregard of the above discussed methodic limitations of indirect measurements. Since the pressure relations on which such indirect measurements are based in the case of ASD with prevailing high flow conditions may be different from those encountered normally some of these relations were analyzed on the basis of the present material The correlations and regression equations elicited in this analysis have been stated in Table 13 The ventricular end diastolic pressure correlates well with mean atrial pressure as has been stated by various authors (Lausom et al 1946 Dexter et al. 1951 Sarnoff and Berglund 1954) and as is also evident from equation No 2 and 5 The correlation of the z point in atrial tracing on $P_{LV\,ev\,I}$ was good too conforming to the report of Braunwald et al. (1961) but there were greater differences in pressure at high $P_{LV\,ev\,ed}$ the z point being slightly higher (Eq No 1 and 4) A similar pattern is seen in the relationship between the z point and mean pressure in the wedge tracings and $P_{LV\,ed}$ (Eq No 6 and 8) In the case of the stasis wave (Nixon and Wooler 1961) its first derivative (time slope) in the left and right atrial and wedge tracings correlated well with the corresponding ventricular slopes of diastasis but in the wedge tracings the first derivative of the stasis wave tended to be slightly low at lower values of the slope in ventricular diastasis There was no evidence of abnormally elevated pressure gradient between the mean wedge pressure and mean left atrial or left ventricular end diastolic pressure which has been reported in ASD by some authors (Rapaport and Dexter 1958 Kaltman et al. 1966) in instances with satisfactory tracings it is true though, that $P_{LV\,ed}$ was slightly lower at higher value of the wedge pressure presenting a difference of 3 mm Hg at 15 mm Hg level and zero difference when the level was 6 mm Hg. On the strength of these observations the right atrial and wedge mean pressures were considered to be satisfactory approximations of the right and left ventricular filling pressures in the present material. When no left ventricular pressure tracing was available as in the case of the postoperative catheterizations the systolic and mean systolic left ventricular pressures were

Table 13 Correlations and regression equations of pairs of different intracardiac pressures (x,y) in 129 cases of ASD utilized in indirect estimation of (y) from (x) RA = right atrium RV = right ventricle PAW = pulmonary artery wedge LA = left atrium LV = left ventricle $\Delta p/\Delta t$ = slope of stasis wave Most records were preoperative ones in Items No 2 and 12 also postoperative values have been included

No	Site of pressure record x	y	Number of pairs	r	$y = a_1 x + a_0$	Standard error of estimate
1	RA *(z)*	RV *(ed)*	129	0 741	y = 0 689 x + 1 85	1 72
2	RA *(mean)*	RV *(ed)*	220	0 873	y = 0 901 x + 1 07	1 28
3	RA *(a)*	RV *(ed)*	114	0 800	y = 0 654 x + 0 92	1 39
4	LA *(z)*	LV *(ed)*	18	0 828	y = 0 773 x + 1 25	1 52
5	LA *(mean)*	LV *(ed)*	55	0 929	y = 0 925 x + 1 22	1 03
6	PAW *(z)*	LV *(ed)*	8	0 764	y = 0 700 x + 2 20	1 48
7	PAW *(mean)*	LA *(mean)*	65	0 811	y = 0 741 x + 0 36	1 51
8	PAW *(mean)*	LV *(ed)*	46	0 783	y = 0 695 x + 0 71	1 69
9	PAW *(a)*	LA *(a)*	9	0 476	y = 0 923 x + 3 95	3 27
10	PAW *(v)*	LA *(v)*	10	0 845	y = 0 725 x + 1 64	1 87
11	PAW *(mean)*	PAW *(a)*	129	0 949	y = 1 235 x + 0 58	1 99
12	PAW *(mean)*	PAW *(v)*	131	0 935	y = 1 350 x + 0 29	3 54
13	RA *(Δp/Δt)*	RV *(Δp/Δt)*	109	0 954	y = 1 014 x + 0 83	1 94
14	LA *(Δp/Δt)*	LV *(Δp/Δt)*	19	0 857	y = 0 856 x + 2 30	4 20
15	PAW *(Δp/Δt)*	LA *(Δp/Δt)*	10	0 930	y = 1 196 x + 1 17	1 73
16	PAW *(Δp/Δt)*	LV *(Δp/Δt)*	7	0 838	y = 0 804 x + 3 60	2 53

estimated from the arterial pressure tracings Such estimated pressure values have been excluded from the calculations of means and of the standard deviations of pressure data but were applied in derivation of other haemodynamic parameters

Comments on interpretation of the dye dilution curves

The dye dilution curves tend towards broadening and lower peak height accordingly as the site of *injection is moved to greater distance from the sampling site* (Hetzel et al 1954) This is particularly to be noted in respect of injection of the dye into a peripheral vein, as compared to central venous injection, independent of the method of recording employed Owing to the circumstance a high proportion of the tracings obtained with peripheral injection may be unreadable However good agreement is reported by some authors between the cardiac output or central blood volume values obtained by Fick s method and those found from peripheral dye dilution curves (Richardson et al 1959 Dendler Rigotti 1961) No systematic difference has been established on comparison of the cardiac output values obtained by the dye dilution technique with peripheral injection with and without flushing with saline and the values determined by means of central vein injection, provided that flushing is used (Bousvara et al 1962) Application of reactive hyperaemia (Bender and Koch 1960 Binder 1964) is a simple means to elicit marked increase of venous flow in the arm its flushing effect is sufficient to produce curves similar to those recorded after central injection (Voegt et al 1965) Peripheral dye injection and recording of the curve with an ear oxymeter has been employed in detection of shunts in congenital cardiac defects (Broadbent and Wood 1954, Woolf et al 1958) The dye curves thus obtained have also been employed as a simple and reliable method for detection of significant residual shunt after cardiac surgery either intraoperatively (Morrow et al 1966) or postoperatively (Oakley et al 1963)

Broadbent and Wood (1954) state that the disappearance time/build up time ratio *(DT BT)* is a good index of the presence of left to-right shunt and that this ratio is abnormal if the shunt is larger than 35 % of the pulmonary flow Carter et al (1960) report that with the aid of two disappearance ratios probably the same sensitivity in detection of a left to right shunt is achieved as with the blood oxygen content method They measured the concentration at the instant corresponding to once or twice the build up time after attainment of the peak concentration to peak concentration ratio ($C_{p\ BT}/C_p$ and $C_{p\ 2BT}/C_p$) and furthermore the lowest concentration systemic recirculation concentration ratio (C_L/C_R) in healthy subjects, in patients with heart disease without shunt or valvular regurgitation, and in patients with left to-right shunt. $C_{p\ 2BT}/C_p$ was less than 0.28 (mean + 2 × S.D) in the cases without shunt and higher than 0.28 in the majority of the cases having a left to right shunt larger than 35 % of pulmonary flow C_L/C_R was less than 0 72 (mean + 2 × S D) in the cases without *shunt, and* it was higher in most of the cases in which a shunt of 10—20 % of pulmonary flow or larger was present. The dye was injected into the pulmonary artery

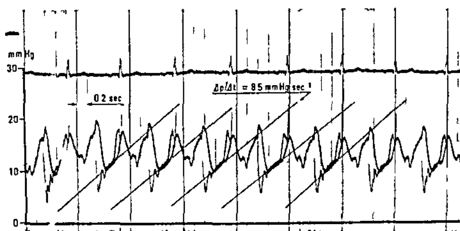


Fig 16 Record of the pulmonary wedge pressure and left ventricular pressure at rest in a case of mild post matic restrictive disease of the heart obtained in simultaneous measurement with two No 8 Cournant cath 125 cm in length The catheter for intravenous catheterization was introduced by the right antecubital vein *that for retrograde catheterization of the left ventricle by the right brachial artery The zero reference point* 10 cm above the table surface The wedge pressure show 80 msec lag of its peaks and 60 msec of the starting p of its upstroke after the corresponding points in the left ventricular tracing The z point is poorly observ In both tracings the slope of the stasis wave ($\Delta p/\Delta t$) is closely equal 8.5 mmHg/sec — $P_{LV\,d} = 14$ mmHg (normal) SVI = 48 ml (normal) DFP = 0 46 sec HR = 53/min D 1 = SVI/$P_{LV_{ed}}$ = 3 4 (low value normal r 8 16 S D 3 80 range 3 8 — 32 9) D 2 = SVI/($\Delta p/\Delta t \times$ DFP) = 16 0 (normal value normal mean 10.38 S D range 3 8 — 32 9) D 3 = $P_{LV_{ed}}/(\Delta p/\Delta t \times$ DFP) = 4 7 (high value normal mean 1 28 S D 0 73 range 0 7—

In the present study the $C_{P\ PAT}/C_P$ and C_L/C_R ratios were calculated from the peripheral dye dilution curves of 16 healthy subjects The upper normal limit thus found (mean + 2 × S D) was 0.33 for C_{P+PAT}/C_P and 0 67 for C_L/C_E In the case of the curves derived from 55 patients of the present series of ASD after surgery in whom no left-to-right shunt was detectable by catheterization the upper limits of 0 33 and 0 77 were found for $C_{P\ PAT}/C_P$ and C_L/C_R respectively Considering values of $C_{P\ PT}/C_P$ higher than 0 40 and C_L/C_R values in excess of 0 85 to be abnormal, these limits were only exceeded in one case in which no shunt was revealed by oxymetry (0 45 and 0 86 respectively being obtained) in all likelihood owing to tricuspid regurgitation. Both ratios displayed on the average slightly higher values in cases with fully surgically corrected defect than in healthy subjects This may be due to larger heart volumes in the former group and perhaps also to slight tricuspid regurgitation, which is probably common in surgically corrected cases of ASD despite absence of clinical and catheter signs Loogen et al 1961)

Calculation of haemodynamic parameters

From the basic flow and pressure data the various parameters were calculated by the following formulae The abbreviations are explained in the Appendix.

Pulmonary and systemic peripheral vascular resistance (R_p and R) $[(\bar{P}_{PA} - \bar{P}_R)/Q_p] \times 80$ (dynes sec cm^{-5}) $[(\bar{P} - \bar{P}_{RA})\ Q\ ] \times 80$ (dynes sec cm^{-5}) The respective indices (R and R) were obtained by multiplying the resistance values with BSA in m²

Stroke volumes or stroke volume indices (SV_R and SV_{LV} SVI_{RV} and SVI_{LV}) Q_p/HR $Q\ /HR$ or Q_{pi}/HR $Q\ _i/HR$ (ml)

Mean systolic ejection rates ($MSER_{RV}$ and MSE SVI_{RV}/SEP_{RV} SVI_{LV}/SEP_{LV} (ml sec^{-1})

Pressure time indices per beat (PTI_{RV} and PT $\bar{P}_{RV} \times SEP_{RV}$ $\bar{P}_{RV} \times SEP_{LV}$ (mm Hg sec)

Stroke work indices (SWI_{RV} and SWI_{LV}) SVI $(\bar{P}_{RV} - P_{RV\ d}) \times 13\,6 \times 10^{-5}$ $SVI_{LV} \times (\bar{P}_{LV} - P_L$ $\times 13\,6 \times 10^{-5}$ (g m)

Ventricular distensibility indices $D\ 1_{RV}$ and D according to Rove et al (1961) $SVI_{RV}/P_{RV_{ed}}$ $SVI_{LV}/P_{LV_{ed}}$ (ml mm Hg^{-1})

Ventricular distensibility indices $D\ 2_{RV}$ and D according to the $\Delta v/\Delta p$ ratio of Feigenbaum e (1966) SVI_{RV}/(*time derivative of RA stasis wa* DFP_{RV}) SVI_{LV}/(*time derivative of LA or w stasis wave* $\times\ DFP_{LV}$) (*ml mm* Hg^{-1}) The techn of determination of the time derivative of st wave is illustrated in Fig 16

Ventricular distensibility indices $D\ 3_{RV}$ and D $P_{RV\ d}$/(*time derivative of RA stasis wave* $\times$ *DFP* $P_{LV\ d}$/(*time derivative of LA or wedge stasis wa* DFP_{LV}) (sec mm Hg^{-1}) D 3 correlates posit on P_d and/or on the plateau of ventricular diast as illustrated by Fig 16

Exercise factor (EF) i e the slope of (l/min) on V_{O_2} (ml/min) in change from ste state at rest to that during exercise

$$EF = (Q \quad - Q \quad)/(V_{O_2} \quad - V_{O_2} \quad)$$

Normal values for 17 healthy subjects are press ed in the Appendix (p 148)

MAIN PREOPERATIVE HAEMODYNAMIC CHARACTERISTICS

The pertinent observations made at right heart catheterization in 129 patients prior to surgery have been compiled in Table 14

Blood oxygen content

The *arterial oxygen saturation* (Sa_{O_2}) before the operation averaged 97.4 % in the group of patients younger than 40 years from which the slightly lower mean of the old age group 95.5 % differed significantly (**) Twelve patients presented values lower than 95 % but higher than 90 % Of these four had an incipient pulmonary vascular disease and five were older than 40 years The two lowest values 88 % and 77 % were recorded in patients with advanced pulmonary vascular disease The 14 patients with reduced arterial oxygen saturation had pulmonary-to-systemic flow ratios ($\dot{Q}_p/\dot{Q}_s$) averaging 2.72 which falls short of the average of the entire series at a statistically significant level (**) Reduced pulmonary venous oxygen saturation was only noted in two out of the 72 cases in which this datum was obtained

The *arteriovenous oxygen difference (AVD)* averaged 4.86 vol % in the young age group and significantly (*) higher 5.15 vol % in the old age group Both these figures even though they are high are still within normal range If the upper limit of normal variation is fixed at 5.5 vol % abnormally great *AVD* was present in 42 patients (33 %) comprising 13 patients older than 40 years (46 % of this age group) and including eight of the 11 patients with atrial fibrillation and ten of the 15 who had pulmonary vascular disease and/or pulmonary hypertension

$C_{\bar{v}O_2}$ increased in all instances by more than 1.0 vol % from *superior vena cava (SVC) to* right atrium (RA) or pulmonary artery (PA) The defect was verified by catheter passage in 86 cases in 81 of these a pressure record was obtained and in five only a sample of the saturated blood

Blood flow

The *systemic blood flow* relative to body surface area (Cardiac index $\dot{Q}_s$) averaged 2.92 in the group of patients younger than 40 years and 2.42 in the old age group There was no statistically significant difference between the groups Both values although low are within the normal range If the lower limit of normal variation is assumed to be 2.0 l/min. there were 17 patients (13 %) whose cardiac index was abnormally low the lowest value was 1.5 l/min Of these 17 patients only three belonged to the group of 40 years or older three of them had incipient pulmonary vascular disease and one presented hyperkinetic pulmonary hypertension Low cardiac index was noted in four out of the 11 patients with atrial fibrillation

The *pulmonary flow* relative to body surface area (Pulmonary flow index $\dot{Q}_p$) varied widely ranging from 2.7 to 17.3 l/min

The *pulmonary-to-systemic flow ratio* ($\dot{Q}_p/\dot{Q}_s$) averaged 3.60 in the group of patients younger than 40 years and 3.27 in the old age group there being no statistically significant difference between the groups This ratio was less than 2.0 in ten cases (7.8 %) between 2.0 and 2.9 in 38 (29.5 %) between 3.0 and 3.9 in 38 (29.5 %) between 4.0 and 4.9 in 26 (19 %) and 5.0 or higher in the rest of the cases (in 17.0 %) Of 40 cases with $\dot{Q}_p/\dot{Q}_s$ less than 3.0 twelve belonged to the old age group (43 %) whereas only two of those having $\dot{Q}_p/\dot{Q}_s$ 5.0 or higher were 40 years or older Of 15 patients with pulmonary vascular disease and/or pulmonary hypertension nine (60 %) had $\dot{Q}_p/\dot{Q}_s$ less than 3.0 The relationship between $\dot{Q}_p/\dot{Q}_s$ and *AVD* in the present series is elucidated by Table 15 Increase of *AVD* beyond the normal range becomes commoner with increasing magnitude of the left-to right shunt

Pressures

The *right atrial mean pressure* ($\bar{P}_{RA}$) averaged 3.8 mm Hg in the group of patients younger than 40 years and 6.3 mm Hg in the old age group the difference being statistically significant (***) If 5 mm Hg is considered to be the upper limit of the normal variation abnormally high P_{RA} was noted in 34 cases (26 %) half of the patients of the old age group presented an abnormally high

Table 14 Haemodynamic findings in 129 patients at rest before surgery

		AVD	S_{O_2}	*Q*	Q_s/Q	$\bar{P}_{RA}$	$\bar{P}_{PA}$	$P_{RV}-P_{PA}$	$\bar{P}$	R_{P_1}	$\bar{P}_{LA}-\bar{P}_{RA}$
Age < 40											
a) Postoperatively recatheterized	*N*	33	33	33	33	33	33	33	33	33	
N = 33	$\bar{x}$	4 86	97 4	2 92	3 38	4 6	20 1	5 2	94 6	144 7	
	S D	1 36	2 8	0 77	1 27	2 4	10 5	5 0	12 1	162 1	
	min	2 9	88	1 5	1 2	0	10	0	68	40	
	max	8 6	100	4 9	6 8	10	65	21	122	980	
b) No postoperative recatheterization	*N*	68	68	68	68	68	68	68	63	67	
N = 68	$\bar{x}$	5 12	97 3	2 58	3 75	3 5	15 9	6 6	90 7	92 5	
	S D	1 11	1 4	0 61	1 21	2 0	4 1	7 5	10 9	56 2	
	min	2 5	94	1 5	1 5	1	9	0	65	28	
	max	7 9	100	4 7	7 7	8	28	40	120	395	
c) Total group	*N*	101	101	101	101	101	101	101	96	101	61
	$\bar{x}$	5 04	97 4	2 69	3 6	3 8	17 3	6 1	92 1	109 7	1 2
N = 101	S D	1 20	2 0	0 68	1 2	2 0	7 1	6 9	11 5	106 9	1 1
	min	2 5	88	1 5	1 2	0	9	0	65	28	0
	max	8 6	100	4 9	4 7	10	65	40	122	980	4
Age ≦ 40											
d) Postoperatively recatheterized	*N*	25	25	25	25	25	25	25	25	25	
N = 25	$\bar{x}$	5 56	95 5	2 46	3 31	6 7	26 0	3 0	108 1	239 1	
	S D	1 20	4 2	0 53	1 38	2 8	9 1	3 6	12 9	209 0	
	min	4 0	77	1 7	1 6	2	11	0	80	59	
	max	7 8	100	3 5	8 0	13	54	12	134	994	
e) No postoperative recatheterization	*N*	3	3	3	3	3	3	3	3	3	
	min	5 1	95	1 8	2 8	1	13	0	85	80	
N = 3	max	5 9	99	2 3	3 1	5	16	5	92	134	
f) Total group	*N*	28	28	28	28	28	28	28	28	28	20
	$\bar{x}$	5 55	95 7	2 42	3 27	6 3	24 9	2 9	106 0	227 2	1 4
N = 28	S D	1 12	4 1	0 53	1 31	3 0	9 2	3 5	13 5	203 6	1 4
	min	4 0	77	1 7	1 6	1	11	0	80	59	0
	max	7 8	100	3 5	8 0	13	54	12	134	994	5
Total series											
N = 129	*N*	129	129	129	129	129	129	129	124	128	81
	$\bar{x}$	5 15	97 0	2 63	3 55	4 4	18 9	5 4	95 2	135 4	1 3
	S D	1 21	2 6	0 67	1 26	2 6	8 3	6 4	13 4	141 8	1 2
	min	2 5	77	1 5	1 2	0	9	0	65	28	0
	max	8 6	100	4 9	8 0	13	65	40	134	994	5
Differences											
a—b	t	1 023	0 237	2 376	2 162	2 573	2 815	0 969	1 640	8 392	
	P(t)	NS	NS	*	*	*	**	NS	NS	***	
c—f	t	2 048	2 960	1 904	1 309	5 156	4 677	2 388	5 416	5 557	0 583
	P(t)	*	**	NS	NS	***	***	*	***	***	NS

Table 15 Relationship between systemic arteriovenous oxygen difference (*AVD*) and pulmonary-to-systemic flow ratio (Q_p/Q) in 129 cases of ASD secundum before surgery

AVD (Vol %)	Q_p/Q		
	<3 0	3 0—4 9	≧5 0
≦ 3 4	3	4	—
3 5 — 4 4	16	10	2
4 5 — 5 4	16	24	4
5 5 — 6 4	8	11	8
6 5 — 7 4	2	10	3
≧ 7 5	3	5	—

Table 16 Relationship between arteriovenous oxygen difference (*AVD*) and the ratio of left atrial *a* wave amplitude and left atrial mean pressure (*a/m*) in 79 cases of ASD secundum before surgery — AF = atrial fibrillation

AVD (Vol %)	*a/m* (LA)			
	1 00 (AF)	1 00—1 99	2 00—2 99	≧3 00
3 0 — 4 9	2	13	3	2
5 0 — 6 9	4	33	8	—
7 0 — 8 9	5	8	—	—
≧ 9 0	—	1	—	—

Table 17 Relationship between heart volume (*HV*) and the ratio of the amplitude of left atrial *a* wave and left atrial mean pressure (*a/m*) — AF = atrial fibrillation

HV (ml)	*a/m* (LA)				
	1 00 (AF)	1 01—1 49	1 50—1 99	2 00—2 49	≧ 2 50
≦ 500	—	3	2	3	—
501 — 600	—	3	4	2	2
601 — 700	—	5	3	1	—
701 — 800	—	2	—	—	—
801 — 900	4	2	1	—	—
≧ 901	6	—	1	—	—

value $\bar{P}_{RA}$ was 10 mm Hg or more in eight cases (6 %) the highest value recorded was 13 mm Hg Six of these eight patients belonged to the old age group Only two of them had slightly elevated diastolic arterial blood pressure and no other apparent ventricular overload occurred in any of them either Among the 15 cases with pulmonary vascular disease and/or pulmonary hypertension there were only three with $\bar{P}_{RA}$ 10 mm Hg or higher and abnormally high values of any degree occurred in only seven of the 15 patients The 11 cases with atrial fibrillation included nine with right atrial pressure higher than 5 mm Hg and six with 10 mm Hg or more

The *left atrial mean pressure* ($\bar{P}_{LA}$) was recorded in 81 cases (63 % of the series) This

Table 14 Haemodynamic findings in 129 patients at rest before surgery

		AVD	S_{O_2}	Q	Q_p/Q	P_{RA}	$\overline{P}_{PA}$	P_{RV} P_{PA}	$\tilde{P}$	R_p	$\overline{P}_{LA}$ P_{RA}
Age < 40											
a) Postoperatively	N	33	33	33	33	33	33	33	33	33	
recatheterized	$\bar{x}$	4 86	97 4	2 92	3 38	4 6	20 1	5 2	94 6	144 7	
N 33	SD	1 36	2 8	0 77	1 27	2 4	10 5	5 0	12 1	162 1	
	min	2 9	88	1 5	1 2	0	10	0	68	40	
	max	8 6	100	4 9	6 8	10	65	21	122	980	
b) No postoperative	N	68	68	68	68	68	68	68	63	67	
recatheterization	$\bar{x}$	5 12	97 3	2 58	3 75	3 5	15 9	6 6	90 7	92 5	
N 68	SD	1 11	1 4	0 61	1 21	2 0	4 1	7 5	10 9	56 2	
	min	2 5	94	1 5	1 5	1	9	0	65	28	
	max	7 9	100	4 7	7 7	8	28	40	120	395	
c) Total group	N	101	101	101	101	101	101	101	96	101	61
	x	5 04	97 4	2 69	3 6	3 8	17 3	6 1	92 1	109 7	1 2
N 101	SD	1 20	2 0	0 68	1 2	2 0	7 1	6 9	11 5	106 9	1 1
	min	2 5	88	1 5	1 2	0	9	0	65	28	0
	max	8 6	100	4 9	4 7	10	65	40	122	980	4
Age ≦ 40											
d) Postoperatively	N	25	25	25	25	25	25	25	25	25	
recatheterized	$\bar{x}$	5 56	95 5	2 46	3 31	6 7	26 0	3 0	108 1	239 1	
N 25	SD	1 20	4 2	0 53	1 38	2 8	9 1	3 6	12 9	209 0	
	min	4 0	77	1 7	1 6	2	11	0	80	59	
	max	7 8	100	3 5	8 0	13	54	12	134	994	
e) No postoperative	N	3	3	3	3	3	3	3	3	3	
recatheterization	min	5 1	95	1 8	2 8	1	13	0	85	80	
N – 3	max	5 9	99	2 3	3 1	5	16	5	92	134	
f) Total group	N	28	28	28	28	28	28	28	28	28	20
	x	5 55	95 7	2 42	3 27	6 3	24 9	2 9	106 0	227 2	1 4
N 28	SD	1 12	4 1	0 53	1 31	3 0	9 2	3 5	13 5	203 6	1 4
	min	4 0	77	1 7	1 6	1	11	0	80	59	0
	max	7 8	100	3 5	8 0	13	54	12	134	994	5
Total series											
N 129	N	129	129	129	129	129	129	129	124	128	81
	x	5 15	97 0	2 63	3 55	4 4	18 9	5 4	95 2	135 4	1 3
	SD	1 21	2 6	0 67	1 26	2 6	8 3	6 4	13 4	141 8	1 2
	min	2 5	77	1 5	1 2	0	9	0	65	28	0
	max	8 6	100	4 9	8 0	13	65	40	134	994	5
Differences											
a—b	t	1 023	0 237	2 376	2 162	2 573	2 815	0 969	1 640	8 392	
	P(t)	NS	NS	*	*	*	**	NS	NS	***	
c—f	t	2 048	2 960	1 904	1 309	5 156	4 677	2 388	5 416	5 557	0 583
	P(t)	*	**	NS	NS	***	***	*	***	***	NS

icant difference (***) between the groups The value of 120 mm Hg was exceeded in four cases only

Representativeness of the group of 58 patients subjected to more detailed haemodynamic study

Among the parameters considered in the foregoing *AVD* S_{O} $P_{sr}-P_{r4}$ and $\overline{P}$ yielded no statistically significant difference between the groups of patients catheterized and not catheterized at the follow up examination while significant differences were elicited in respect of $\overline{P}_{s4}$ (*) $\overline{P}_{r4}$ (***) and R_{p} (***) The differences were rather minimal all the same as can be seen from Table 14 this even applies to R_{pi} if one takes into account the large magnitude of the standard deviation in the group of patients younger than 40 years and not subjected to catheterization which is due to the fact that in this group there was only one patient with OPH

Indicator dilution tests

As has been stated in the foregoing indicator dilution tests were carried out in the majority of the present cases They were only qualitatively interpreted to serve as a diagnostic aid in evaluating the magnitude of the left to right shunt presence of a right-to-left shunt and anomalies of the pulmonary veins The observations which were made are largely consistent with those reported by Samet et al (1962) and are not analyzed in this connection

Comments on preoperative haemodynamic findings

Compared with some other adult series (e g Craig and Selzer 1968) arterial desaturation was not commonly encountered in the present series S_{O_2} was normal in 91 % of the cases its reduction whenever present was only moderate and mostly associated with higher age and with pulmonary vascular disease In about half of the cases presenting arterial desaturation the origin of venous blood admixture remained indeterminate in lack of a pulmonary venous sample Of the other half of such cases the pulmonary blood was slightly undersaturated in two Both these patients had pulmonary vascular disease and only moderate pulmonary blood flow Caval blood admixture at atrial level was demonstrated by the dye technique in many cases but not in all those displaying arterial desaturation Beller (1967) has shown that ventricular asynchronism due to complicating left bundle branch block or to ventricular preexcitation in ASD may elicit arterial desaturation by causing temporary reversal of the shunt at the left ventricular presystole It is not impossible that also some other type of asynchronism operating at the rapid filling phase may occasionally be involved Three cases of ASD have been seen in our laboratory in which marked arterial desaturation was associated with moderate left-to right shunt normal $S_{r4_{O_2}}$ and absence of pulmonary hypertension These ca es pre sented the common feature of prolonged QRS complex due to right bundle branch block of exceptional high degree In the present series no such phenomena were observed but in six out of eight cases with arterial desaturation without pulmonary hypertension the QRS complex was in excess of 120 msec whereas the incidence of right bundle branch block amounted to 20 % in the entire series

Q is a rather inconstant and therefore unsatisfactory measure for u e as a standard of normal blood flow in respect of individuals of different sex age and body size under conditions of cardiac catheterization in which often basal state does not prevail because it is a linear function of $\dot{V}_{O_2}$ in the resting subject In contrast the systemic arteriovenous oxygen difference *(AVD)* in resting subjects is a rather cons ant measure wich is independent of $\dot{V}_{O_2}$ basal metabolic rate and body size (Reeves et al 1961) In the present series these parameters both displayed a borderline low normal value on the average abnormally low values being common The normal reference values (see Appendix) were ob ained by calculations in which the superior caval blood was u ed for mixed venous blood for which reason the objections are not valid in the comparison instituted here which Marshall et al (196-) made in respect of different venous sampling in ASD and in normal cases The present findings agree with those of Dex er (1956) and also with the observations made by Sterstein and Efskind (1963) who found

reduced $\dot{Q}$ in 12 % of their cases Evidence of abnormally low systemic circulation particularly in old subjects with ASD has been reported by several authors (e g Davies and Gazetopoulos 1966 Petersson 1967, Craig and Selzer 1968) In the present series similar prevalence of low Q_i was noted in both age groups but the frequency of low *AVD* was distinctly higher than that of low Q in both age groups particularly in the old patients Many authors have considered the cardiac index or cardiac output to be normal in uncomplicated ASD (Brotmacher and Deuchar 1956 Weidman et al 1957 Marshall et al 1962) In the present series reduced Q_i was usually associated with some complicating disorder such as pulmonary vascular disease atrial fibrillation or both but in uncomplicated cases it was mostly within the normal range In contrast only three of the 29 younger patients with high *AVD* had any complications suggesting that hypokinetic systemic circulation is a fairly common feature in ASD regardless of the presence or absence of complications

Elevated $\overline{P}_{RA}$ was only noted in one-quarter of the present cases and it was more common in the old age group particularly in patients with atrial fibrillation Only 38 % of the patients with elevated $\overline{P}_{RA}$ had a history of circulatory congestion but even this is a percentage twice as high as in the patients with normal $\overline{P}_{RA}$ In 18 % of the said patients the diastolic arterial blood pressure was elevated contrasted to 5 % of those with normal $\overline{P}_{RA}$ This figure is no higher than might be expected from the prevalence of elevated blood pressure in the older patients of the series and from the proportion of cases with increased $\overline{P}_{RA}$ among them $\overline{P}_{RA}$ was normal in the rest of the patients with arterial hypertension No other kind of haemodynamic left ventricular burden or apparent pathological left ventricular condition as postulated by Dexter (1966) and by Tikoff et al (1966) was detected in the cases with elevated $\overline{P}_{RA}$ either

One of the salient characteristics of the atrial pressures found in ASD is slightly elevated $\overline{P}_{RA}$ and reduced $\overline{P}_{LA}$ which both were also apparent in the present observations Normally there is a pressure gradient about 4—5 mm Hg in magnitude across the intact atrial septum (Braunwald et al 1961) In the present series of ASD patients the mean interatrial pressure gradient was only 1 3 mm Hg and no gradient was measurable in one-fourth of the cases The magnitude of the gradient had no relation to the size of the defect as established at operation Cohn et al (1967) have recently found that the diameter of the defect averages as much as 3 cm in spite of existing interatrial pressure gradient in the average amount of 1 8 mm Hg Levin et al (1968) found in their careful study of atrial pressure-flow dynamics in ASD in 26 children that $\overline{P}_{LA}$ was 5 8 mm Hg and $\overline{P}_{RA}$ was 5 1 mm Hg while the respective $P_{LV_{ed}}$ and $P_{RV_{ed}}$ were 10 and 6 mm Hg They observed two peaks of the left-to right shunt during the heart cycle one during the late ventricular systole and early diastole and another during the auricular systole The corresponding peaks of interatrial pressure gradient they attributed mainly to the inertial component of the shunting blood mass the resisother during the auricular systole The corresponding peaks of interatrial pressure gradient for the poor relationship established between the interatrial pressure gradient and the size of the defect

One of the determinants of mean atrial pressure in an intact animal is the strength of atrial contraction At reduction of the atrial contraction force the mean atrial pressure rises and *vice versa* (Sarnoff and Mitchell 1961) The same applies to the human mean atrial pressure e g at change from normal sinus rhythm to atrial fibrillation (Braunwald and Frahm 1961) Accordingly the ratio of the amplitude of the atrial *a* wave and the atrial mean pressure *(a/m)* may be considered a kind of rough measure of atrial contractility purely in terms of pressure and disregarding the potential variability of atrial dimensions This ratio is unity in atrial fibrillation and its value is obviously greater accordingly as the share of the atrial booster pump function in the overall function increases In the present series of ASD cases the left atrial *a/m* ratio tended to be high in connection with low interatrial gradient and small *AVD* Since in all likelihood the interatrial gradient in the presence of a significant left-to-right shunt is rather more strongly related to the magnitude of the shunt volume higher *a/m* ratios may be assumed to be associated with small left-

to right shunt and furthermore with small *AVD* There was in fact in spite of the limitations of the method which have been mentioned a slight trend of inverse relationship between *AVD* and the *a/m* ratio in the present study *which might suggest some connection* between hypokinetic systemic circulation and defective atrial function in ASD It seems likely that this relationship is in some part due to the large heart volume of certain patients with high *AVD* since *it is reasonable* that the a/m ratio is influenced by the atrial dimensions according to Laplace's law This is manifested in the slight inverse relationship established between the *a/m* ratio and the heart volume in Table 17 (p 93)

The atrial function may be considered normally to consist of three components as follows (1) The booster pump function (e g Linden and Mitchell 1960 Braunwald and Frahm 1961) which normally accounts for 20 % (2 % —39 %) of the total atrial work (Grant et al 1964) *(2) The reservoir function of the atria* consists of accumulation of pulmonary or systemic venous blood during the ventricular systole in the atrium and of its discharge into the ventricles at their rapid filling phase Its *proportion of the total left atrial work is* considerable averaging about 80 % (Grant et al 1964) (3) The concept of the pipe function of the atria characterizes the passive flow-conducting role of the atrium at ventricular filling (*Grant et al 1964*) *The compliance* of the left atrial-pulmonary venous system is considerably less than that of the right atrium and associated veins (Cournand et al 1947 Little et al 1949) similarly the left *ventricular distensibility is inferior to that of* the right ventricle Therefore in the presence of a large atrial septal defect the reservoir and booster pump functions in particular may be expected to be affected by leakage to the *more compliant right side of the heart* the pipe function presumably being affected least of all

In addition to this anatomical reason for poor efficiency of the atrial activity there are indications of impairment of atrial contraction in terms of pressure with increasing age and heart volume In cases with total atrial contraction failure as in atrial fibrillation the mean atrial pressure (or at least $\overline{P}_{LA}$) must rise in ASD to the level of the left ventricular filling pressure which may normally be 10—14 mm Hg without implication of left ventricular abnormality There is consequently reason to assume that in many cases impaired atrial function may be responsible for elevated $\overline{P}_{PA}$ in absence of abnormality in the left ventricular function *As a matter of fact* in the present series increased $\overline{P}_{PA}$ was not reasonably attributable to left heart overload in any but two cases with very moderate arterial hypertension

Increased R_{p}, was markedly more prevalent among the patients aged 40 years or older than in the young age group The prevalence of pulmonary hypertension and/or pulmonary vascular disease in percent was 0 in the second decade of age 2 4 % in the third 8 6 % in the fourth 33 % in the fifth and 50 % in the sixth decade Inceasing *frequency of pulmonary* hypertension with increasing age is thus obvious a sudden increase by a factor of 4 being noted at the age of 40 years This is in accordance e g with the observations of Rocksetb (1968) *The sex ratio of the patients with pulmonary* vascular complications was the same as in the entire series

FOLLOW-UP EXAMINATIONS FOR DETECTION OF RESIDUAL SHUNT

Findings

Recatheterization at follow up was performed in 58 cases In 57 instances sufficient oxymetric data were obtained which enabled the right *atrial and pulmonary arterial blood oxygen* contents to be judged in *view of detection of* a potential residual shunt

The difference between $C_{ar_{O}}$ and $C_{PA_{O}}$ exceeded 1 0 vol % in two instances (1 2 and 1 1 vol % respectively) and differences higher than 1 5 vol % were noted twice (1 6 and 1 8 vol %) *In one further case the difference* between $C_{svc_{O}}$ and $C_{PA_{O}}$ was greater than 1 5 vol % *while the difference between* $C_{svc_{O}}$ *and* $C_{PA_{O_2}}$ was normal In three of these five cases *no single high oxygen saturation value was found in the right atrium and the* $C_{ra_{O_2}}$ was *amply above* $C_{svc_{O}}$ and high enough to account for the elevated values in the right atrium and *pulmonary artery as the result of admixture* The dye dilution curves appeared com-

pletely normal in these three cases showing a C_{P+IST}/C_P ratio lower than 0.40 and a C_L/C_R ratio less than 0.85. In the other two cases C_{HCO} was only slightly above C_{HCO_2} and in both cases some very high oxygen saturation readings were obtained from the right atrium. Residual shunt was considered highly probable in these two cases and calculation of its magnitude yielded the value of 30 % and 15—20 % of $\dot{Q}_P$. The C_{P+IST}/C_P ratio was 0.56 and 0.30 respectively and the C_L/C_R ratio was over 1.0 in the former case and 0.88 in the latter one, or both ratios thus having an abnormal value.

In 55 cases in which no residual shunt was indicated by the oxymetric data the C_{P+IST}/C_P ratio exceeded the value of 0.40 in one case only (0.45), in which C_L/C_R was 0.86. C_L/C_R ratios higher than 0.85 were found in five of the 55 cases (0.90, 0.87, 0.87, 0.86, 0.89), but the C_{P+IST}/C_P ratio was simultaneously abnormally high only in the single above-mentioned case. Simultaneous false abnormally high values of both dye curve ratios were thus noted in one single case only. As has been said before this was probably due to slight tricuspid regurgitation.

72 patients were only examined by means of the dye dilution curve without recatheterization. Owing to technical faults the number of satisfactory tracings enabling the C_{P+IST}/C_P ratio to be calculated was 68, while C_L/C_R could be calculated in 66 cases. In all but one of 68 instances C_{P+IST}/C_P was normal. C_L/C_R was higher than 0.85 in two cases (0.87 and 0.89), both of which yielded C_{P+IST}/C_P 0.24. Early recirculation of a small left-to-right shunt was visible in the dye curve in one case in which C_{P+IST}/C_P was 0.42 and C_L/C_R was 0.92, i.e. both abnormally high.

The inference was drawn from these findings that a postoperative residual left-to-right shunt was present in three cases, which had a magnitude of 10—15 %, 20 % and 30 % of Q_P respectively.

Comments

Overlapping of the oxymetric and dye dilution data must reasonably exist between cases with small (less than 30 % of $\dot{Q}_P$) left to right shunt at atrial level and without shunt (Carter et al. 1960). Since the oxymetric and the dye dilution method both possess a sensitivity of the same order, no information is obtainable on the distribution of the dye curve parameters in borderline shunt cases by comparing mutually the dye dilution and oxymetric data. The frequency distributions of the C_L/C_R values were similar both in the recatheterized and not recatheterized groups. Slight dissimilarity was noted in the distributions of the C_{P+IST}/C_P values, which present a slightly higher mean in the latter group. When 13 cases were excluded from the latter group, similar distribution was obtained (mean 0.22, S.D. 0.06). Only one of these 13 cases presented a C_{P+IST}/C_P ratio higher than 0.40, and the C_L/C_R ratio was less than 0.85 in all 13. There is thus good reason for the assumption that the cases not subjected to recatheterization (one single case with apparent residual shunt excluded) as a group did not either differ significantly from the group in which the oxymetric and dye examinations had both established absence of a shunt.

The observations of Carter et al. (1960), and likewise the present limited data concerning the dye disappearance parameters in small left-to-right shunts suggest that it is possible for any haemodynamically significant left-to-right shunt to be discriminated with high degree of probability by the dye dilution method.

HAEMODYNAMIC FINDINGS AT FOLLOW-UP EXAMINATION IN 58 PATIENTS

Altogether 58 patients were recatheterized after surgery. Of them 33 were younger than 40 years and 25 were 40 years or older. The male patients number 18 and the female patients 40, of whom 7 and 18 respectively belonged to the group of patients aged 40 years or older. Observations both at rest and during exercise were made at follow-up in 53 instances. A detailed analysis of the haemodynamic findings recorded in these cases is presented in the following.

Blood flow

Oxygen uptake (V_{O_2}) — Preoperatively V_{O_2} was in the entire recatheterized series 216.2 ml/min (S.D. 30.4); the values found at rest

and during exercise after surgery were 229 3 ml/min (S.D 37 5) and 1099 5 ml/min (S.D 236 2) respectively At the preoperative catheterizations V_o averaged 108.5 % of predicted basal value (S.D 8 9) and at the recatheterizations 110 1 % of predicted value (S.D 9 9) The predicted value was exceeded by more than 20 % in eight cases at the preoperative and in 11 at the postoperative examinations

The average values related to body surface area *(BSA)* are presented in Table 17 The V_{o_2} relative to *BSA* in the entire recatheterized series was preoperatively slightly but significantly (*) higher than postoperatively at rest it was significantly lower in the females than in the males both preoperatively and postoperatively (** and *** respectively) The increase in V_{o_2} per *BSA* during exercise was slightly less (*) in the old than in the young age group

Heart rate (HR Table 18) — *HR* averaged preoperatively 77 6 per minute in the entire recatheterized series the rate of 100 per minute was only exceeded in five cases The average value at rest after surgery 72 6 is significantly lower (*) there were only two values higher than 100 During exercise *HR* averaged 142 9 per minute and there were eight instances in which it exceeded 140 and only five in which it was over 150 No significant differences were established between the age or sex groups In most cases thus the *HR* response to exercise in supine position was in the order which could be expected on the basis of the load chosen In one case with atrial tachycardia and 2 1 A—V block the block disappeared in the course of exercise resulting in a ventricular rate of 210 per minute In two other cases with an unexpected rise in *HR* atrial fibrillation was present On the average *HR* during exercise related to V_{o_2} was slightly lower in the group of patients younger than 40 years than in the older group the older patients thus displayed slightly steeper slope of change of *HR* with V_o

Mixed venous blood oxygen saturation ($S_{\bar{v}_{O_2}}$, Table 18) — In the entire recatheterized series ($S_{\bar{v}_{O_2}}$ averaged preoperatively 66 5 % and it was significantly (***) higher at rest at recatheterization i e 73 6 % This difference was significant in both age groups (***), but in the older age group the value was lower both before and after surgery than in the group of patients younger than 40 years (*) The reduction of $S_{\bar{v}_{O_2}}$ attendant on exercise at recatheterization was more pronounced in the group of patients aged 40 years or older than in the younger group (***)

Arterial blood oxygen saturation (S_{O_2}, Table 18) — S_{O_2} was normal on the average preoperatively in the entire recatheterized series and in its subgroups and likewise at recatheterization at rest as well as during exercise Preoperatively five younger and six older patients displayed reduced S_{O_2}, atrial fibrillation was present in four of them and pulmonary vascular involvement in five All but one of the patients who had residual left-to-right shunt and pulmonary hypertension displayed normal S_{O_2} after surgery Slightly reduced S_{O_2} (lowered to 93—94 %) was furthermore observed at recathe erization in five cases which had preoperatively presented normal saturation The saturation values showed some further decrease during exercise in these cases Two of them had obstructive pulmonary hypertension and one had hyperkinetic pulmonary hypertension prior to surgery The dye dilution curves did not demonstrate right-to-left shunt at atrial level in any of these cases

Arteriovenous oxygen difference (AVD Table 18) — *AVD* averaged 5.2 vol % preoperatively in the entire recatheterization series at recatheterization it was significantly smaller 4.2 vol % (***) Both values are within normal range The difference between the age groups is statistically significant both before and after surgery (* and *** respectively) with lower values in the old age group no statistically significant differences were noted between the sexes *AVD* was preoperatively slightly higher than normal (i e in excess of 5 5 vol %) in 20 instances while there were only six such cases at recatheterization of which four belong to the group of patients aged 40 years or older

Exercise elicited an average change of 4 7 vol % in *AVD* The subgroups did not differ significantly in this respect from each other although during exercise *AVD* was

Table 18 Oxygen uptake *(V_{O_2}/BSA)* heart rate *(HR)*, mixed venous blood oxygen saturation *($S_{\bar{v}O_2}$)* arterial blood oxygen saturation *(S_{aO_2})* and arteriovenous oxygen difference *(AVD)* in the group of patients subjected to recatheterization at follow up — 1R = before operation, at rest, 2R = at follow up at rest 2F = at follow up during exercise 2RFΔ = difference of values during exercise and at rest (at follow-up)

		V_{O_2}/BSA			HR			$S_{\bar{v}O_2}$			S_{aO_2}			AVD			
		1R	2R	2E	1R	2R	2E	1R	2R	2E	1R	2R	2E	1R	2R	2E	2REΔ
MALES	$\bar{x}$	131 8	143 7	681 7	73 8	78 7	127 2	63 7	73 6	47 1	95 9	95 7	95 5	5 3	4 4	9 5	5 1
	S D	7 8	14 4	89 8	10 1	18 7	30 4	0 7	6 4	6 7	3 1	2 4	1 2	1 2	1 1	1 6	1 2
FEMALES	$\bar{x}$	127 7	129 0	674 6	77 1	72 1	123 9	68 7	73 6	45 1	95 9	96 8	93 5	5 1	4 1	8 6	4 5
	S D	9 9	9 0	124 1	15 3	15 2	19 1	8 7	5 1	8 0	3 8	1 3	1 4	1 4	0 9	1 2	0 9
AGE	$\bar{x}$	130 3	133 6	673 6	77 6	72 2	122 5	67 9	75 0	49 7	97 4	96 2	93 5	4 9	3 8	8 3	4 6
	S D	10 3	13 5	106 3	15 6	11 6	15 9	9 0	5 3	6 3	2 8	2 0	1 2	1 4	0 8	1 0	0 9
<40	r		0 639	0 021		0 485	0 413		0 249	0 090		0 562	0 554		0 371		0 460
	t		1 802	27 692		2 180	18 171		4 415	17 966		2 928	1 023		4 756		26 300
	P(t)		NS										NS				
AGE	$\bar{x}$	129 4	133 5	598 0	77 6	73 2	127 9	62 4	71 7	41 4	95 5	96 7	95 9	5 6	4 7	9 6	4 8
	S D	9 3	12 2	119 7	17 5	16 5	19 4	8 3	5 3	8 6	4 4	1 5	1 6	1 2	1 0	1 4	1 2
≥40	r		0 455	0 278		0 79	0 309		0 357	0 406		-0 144	0 655		0 494		0 523
	t		1 784	19 042		1 041	15 017		5 742	22 381		1 237	2 534		4 018		19 345
	P(t)		NS			NS						NS					
ALL	$\bar{x}$	129 9	133 5	649 8	77 6	72 6	124 9	65 5	73 6	46 1	95 6	93 4	96 2	5 2	4 2	8 8	4 7
	S D	9 8	12 8	117 4	16 3	13 8	22 8	9 0	5 5	7 6	3 6	1 8	1 5	1 4	1 0	1 4	1 02
	r		0 568	0 12		0 343	0 469		0 356	0 353		0 174	0 582		0 480		0 613
	t		2 530	31 684		2 192	18 926		7 076	26 475		0 408	0 981		5 999		30 204
	P(t)											NS	NS				
M/F	t	2 941	3 994	1 860	0 318	0 502	0 402	1 012	0 003	0 647	1 057	1 828	2 604	0 557	1 015	1 972	1 752
	P(t)			NS	NS	NS	NS	NS	NS	NS	NS	NS		NS	NS	NS	NS
<40/≥40	t	0 348	0 030	2 391	0 000	0 258	0 310	2 419	2 348	4 628	1 889	1 088	1 473	2 047	3 695	3 777	0 668
	P(t)	NS	NS		NS	NS	NS				NS	NS	NS				NS

Table 19 Pulmonary flow $(\dot{Q}_p)$ pulmonary flow index $(\dot{Q}_{p_i})$ systemic flow $(\dot{Q})$ systemic flow index $(\dot{Q}_i)$, exercise factor $(\Delta Q/\Delta V_{O_2} \times 10^5)$ pulmonary to systemic flow ratio $(\dot{Q}_p/\dot{Q})$ right ventricular stroke volume (SV_{RV}) and stroke volume index (SVI_{RV}) and left ventricular stroke volume (SV_{LV}) and stroke volume index (SVI_{LV}) in the group of patients subjected to recatheterization at follow up — Symbols 1R 2R 2E and 2REΔ as in Table 18

		Q_p	Q_{p_i}	Q				Q_i			$\Delta Q/\Delta V_{O_2}$	Q_p/Q	SV_{RV}	SVI_{RV}	SV_{LV}				SVI_{LV}		
		1R	1R	1R	2R	2E	2REΔ	1R	2R	2E	$\times 10^5$ 2RE	1R	1R	1R	1R	2R	2E	2REΔ %	1R	2R	2E
MALES	$\bar{x}$	16.5	9.2	4.8	6.4	13.7	7.0	2.7	3.5	7.3	692.7	3.5	207.6	115.6	59.7	89.2	109.3	22.[illegible]	33.5	47.7	58.3
	SD	6.4	3.4	1.1	1.6	2.4	1.9	0.7	0.6	1.3	153.8	1.2	84.1	46.0	16.6	21.2	27.9	30.6	10.1	10.6	14.8
FEMALES	$\bar{x}$	13.6	8.6	4.3	5.5	12.1	6.6	2.7	3.4	7.3	800.6	3.3	185.3	114.1	57.1	77.8	97.2	26.0	38.1	47.6	59.3
	SD	5.3	3.4	1.2	1.2	2.8	2.4	0.7	0.8	1.8	149.6	1.4	78.8	49.2	15.5	18.1	21.8	25.3	9.9	12.0	13.6
AGE	$\bar{x}$	15.1	9.4	4.7	6.1	13.8	7.5	2.9	3.7	8.2	832.2	3.4	206.8	125.3	60.9	85.4	115.5	30.5	38.1	51.4	66.7
	SD	5.3	3.2	1.3	1.5	2.8	2.3	0.8	0.8	1.5	144.4	1.3	86.4	51.4	16.6	18.2	23.0	27.6	10.2	11.0	11.1
<40	r				0.446	0.577			0.428	0.465			0.235	0.284		0.403	0.439			0.430	0.375
	t				5.426	18.386			5.394	18.549			8.302	8.591		7.384	6.527			6.697	7.075
	P(t)																				
AGE	$\bar{x}$	13.8	7.9	4.2	5.4	11.0	5.6	2.5	3.0	6.1	689.0	3.3	173.0	100.3	53.9	76.0	86.4	17.9	31.5	42.7	49.1
	SD	6.3	3.5	1.1	1.2	1.8	1.7	0.6	0.5	1.1	137.0	1.4	68.7	39.4	13.9	20.6	17.2	24.1	8.3	10.3	10.1
<40	r				0.591	0.443			0.462	0.218			0.157	-0.007		0.184	0.439			0.065	0.457
	t				5.747	16.265			4.3.2	13.519			7.075	7.060		4.882	2.532			4.339	2.945
	P(t)																				
ALL	$\bar{x}$	14.5	8.8	4.5	5.8	12.5	6.7	2.7	3.4	7.3	770.1	3.4	192.2	114.6	57.9	81.3	100.6	24.9	35.3	47.6	59.0
	SD	5.8	3.4	1.2	1.4	2.8	2.2	0.7	0.8	1.7	157.2	1.3	80.4	47.8	15.7	19.7	24.1	26.6	9.9	11.5	13.8
	r				0.516	0.595			0.311	0.560			0.241	0.261		0.354	0.509			0.385	0.554
	t				7.674	21.461			7.150	19.990			10.824	11.056		8.684	6.458			7.864	6.913
	P(t)																				
M/F	t	1.680	0.622	1.557	2.132	2.082	0.639	0.000	0.527	0.000	2.318	0.557	0.952	0.112	0.563	1.980	1.508	0.393	0.904	0.035	0.236
	P(t)	NS	NS	NS			NS	NS	NS	NS		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<40/≥40	t	0.832	1.677	1.585	1.975	4.416	3.458	2.160	4.091	5.879	3.684	0.278	1.659	2.097	1.746	1.809	4.545	1.770	2.610	3.002	6.130
	P(t)	NS	NS	NS	NS							NS	NS		NS	NS		NS			

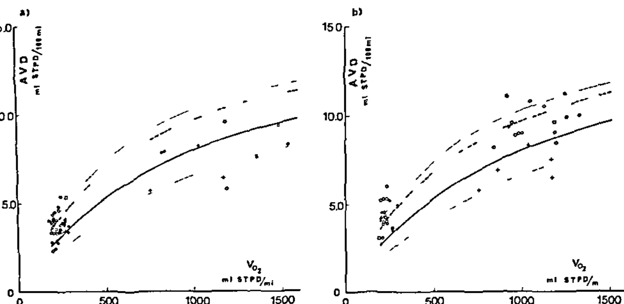


Fig 17 Arteriovenous oxygen difference observed at rest and during exercise at follow up plotted over oxygen uptake for patients of the young (Fig 17 a) and old (Fig 17 b) age group — ● Male o Female patients □ Cases with minor residual shunt + Normal control subjects Heavy line Regression in normal young subjects thin dotted lines its range heavy dotted line regression in normal old subjects (see text)

clearly lower in the old age group (***) As plotted against V_o the *AVD* changes approximately obeyed the normal hyperbolic regressions for healthy young individuals (Ekelund and Holmgren 1967) in the young age group (Fig 17 a) and that for old healthy male subjects (Strandell 1964) in the old age group (Fig 17 b) the only difference being the higher level of the curvilinear pattern in the old subgroup In both age groups the values were postoperatively at rest mostly above the normal regression line whereas during exercise the scatter conformed better with the normal regression

Pulmonary flow ($\dot{Q}_p$) *and pulmonary flow index* ($\dot{Q}_{pi}$ Table 19) In the total recatheterized series Q_p was preoperatively 14 5 l/min on the average and Q_p was on the average 8 8 l/min The values were slightly although not significantly lower in the old age group than in the young age group Taking into consideration the magnitude of the methodic error at follow up there were 2 cases in which the calculations yielded a value of Q greater than Q and in no case less than $\dot{Q}$

Systemic flow ($\dot{Q}$) *and systemic flow index* (Q_i Table 19) In the total recatheterized series $\dot{Q}$ was preoperatively 4 5 l/min and postoperatively at rest significantly (***) higher i e 5 8 l/min A similar change (***) can be seen in the values of $\dot{Q}_i$ In the old age group both Q and Q_i were slightly lower than in the young age group the difference in $\dot{Q}_i$ being statistically significant both preoperatively (*) and at recatheterization (***) The value of Q_i was abnormally low (i e less than 2 0 l/min) preoperatively in 7 cases 6 of them being 40 years of age or older

Exercise caused in Q_s at follow-up an average increase of 6 7 l/min The rise was similar in both sexes but significantly greater (**) in the young age group As plotted against V_o (Figs 18 a and b) Q_s was at follow-up catheterization in the majority of cases below the normal regression line presented for healthy young men and women by Ekelund and Holmgren (1967) The values of five control subjects also display the same level but these were sedentary untrained individuals Of the values

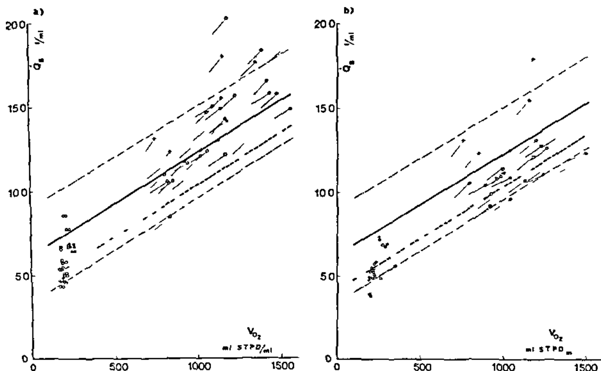


Fig 18 Systemic blood flow found at rest and during exercise at follow up plotted over oxygen uptake for patients of the young (Fig 18 a) and old (Fig 18 b) age group — • Male o Female patients □ Cases with minor residual shunt + Normal control subjects Heavy line Regression in normal young subjects thin dotted lines its range heavy dotted line regression in normal old subjects (see text)

obtained for patients aged 40 years or older at rest the majority are below the normal regression line of healthy old men presented by Strandell (1964) The values of $\dot{Q}$ plotted over V_{o_2} referring to exercise are scattered well inside the normal range in both age groups It is thus noted that at rest Q presented a hypokinetic pattern both before and after surgery while it behaved normally during exercise at follow-up

The slope of $\dot{Q}$ over V_o at transition from rest to exercise ($\Delta Q / \Delta V_{o_2}$) averaged 0 077 in the entire recatheterized series which constitutes a low normal value in our laboratory Expressed in terms of the exercise factor (Wade 1959) this slope averaged 770 1 in the entire series of recatheterized patients the values in the group of patients younger than 40 years and in the older group being 832 2 and 689 0 respectively with a statistically significant difference (***) between the age groups The exercise factor was lower in the male than the female subseries (*) If 600 is considered to be the lower limit of its normal variation there were six patients with an abnormally low factor which ranged from 421 to 554 of these five were 40 years of age or older and only one of them had atrial fibrillation

Stroke volume (SV) and stroke volume index (SVI Table 19) — The right ventricular stroke volume (SV_{RV}) averaged preoperatively 192 2 ml in the entire catheterized series and SVI_{RV} 114 6 ml This latter value was lower in the group of patients aged 40 years or older than in the younger group (*) The left ventricular stroke volume (SV_{LV}) averaged preoperatively 57.9 ml in the entire series and SVI_{LV} averaged 35 3 ml both of which are normal values The values found after surgery were 81 3 and 47 6 ml respectively The change in SV_{LV} and SVI_{LV} from the preoperative to postoperative value was statistically highly significant (***) in the total series and in

both its age groups SVI_{LV} was significantly (**) lower in the old age group than in that of the younger patients both before and after surgery

During exercise at follow-up catheterization SV_{LV} *(SV)* increased by the average of 24 9 % from the value at rest the increase was more pronounced in the group of patients younger than 40 years than in the older group (***) *SV* of seven patients remained unchanged or suffered slight reduction four of them belonged to the old age group and all seven had normal sinus rhythm In one case with chronic atrial tachycardia and 2 1 A-V block *SV* was lowered by 27 % owing to marked tachycardia induced by disappearance of the block during exercise

Comments on the blood flow parameters — Basal circulatory conditions are not usually encountered during heart catheterization The findings of normal average heart rate and of 10 % excess over the predicted basal V_{o_2} at rest both preoperatively and postoperatively approximate those reported from some normal and pathological series (e g Jonsson et al 1957 Granath et al 1964 Petersson 1967) The relative V_{o_2} (per *BSA*) was not different in the present series from that of the controls at rest Owing to individual variability in mechanical efficiency the circulatory parameters during exercise were not related to the external work level but they were related to the level of V_{o_2} The V_{o_2} value related to *HR* was normal both before and after surgery During exercise the amount of V_{o_2} per beat (that is the oxygen pulse) was on the average slightly lower in the group of patients aged 40 years or older, despite the higher *AVD* of this group than in the younger patients which is consistent with the finding of smaller *SV* during exercise in the same group

The reduced S_{o} observed preoperatively in five cases was found after surgery to have normalized in all but one case in which residual shunt was present S_{o_2} normally increases slightly during exercise in healthy young subjects (Bevegård et al 1960 1963) as well as in old males (Granath et al 1964) Such an effect of exercise was only occasionally noted in the present series while in some patients most of them with pulmonary vascular involvement a small decrease of S_{o_2} took place during exercise

In both age groups of the series there ensued on operation a significant increase of $S_{\bar{v}_{O}}$, reduction in *AVD* increase of *Q* and increase of *SV* Although most values were within normal range both before and after surgery distinct change from low normal values toward normal values was evident on the average However the hypokinetic systemic circulatory pattern manifested preoperatively in both age groups but especially in the old age group could still be seen at follow-up Normal circulatory response was observed on the average at exercise in both age groups as judged from the changes in *AVD* and of *Q* related to V_o These observations are largely consistent with those reported by Petersson (1967) in his series of uncomplicated ASD cases except as regards changes in *SV* during exercise which were more marked in the present series Of eight patients with preoperative elevated $\bar{P}_{RA}$ of 10 mm Hg or higher level haemodynamic data recorded during exercise after surgery were available in seven cases Normal *AVD* at rest before exercise was found in five of these all seven presented normal change of *AVD* during exercise all seven had a normal exercise factor, and *SV* showed greater or lesser increase (ranging from 9 to 81 %) in all of them after commencement of work In 11 of the 14 cases with signs of pulmonary vascular disease in the recatheterized series exercise data were available In three of them *AVD* was slightly reduced at rest but the cardiac index was normal in all of them and all presented normal slope over V_o of the change in *AVD* and of *Q* during work with the exception of one patient with slightly reduced exercise factor (554) Only two of the 14 patients with pulmonary vascular disease showed slight reduction of *SV* during exercise (by 4 % and 16 % respectively) while in the rest an increase ranging from 4 % to 71 % was noted

Healthy old subjects have usually higher *AVD* at rest than younger individuals and their *Q* is lower at rest but both parameters have a similar slope over V_{o_2} during work in young as well as old subjects (Granath and

Strandell 1964 Julius et al 1967) Healthy untrained male subjects aged 40—60 years display a trend of relatively high Q and SV during light work with reduction of the values at submaximal work (Hanson et al 1968) The slope of Q, over V_{o_2} (and the magnitude of the exercise factor) naturally also depends on the level of V_{o_2} at rest and its steepness has been found to be greater in old subjects accordingly as the circulatory state is closer to basal conditions (Granath and Strandell 1964) In the present series the patients with low exercise factor at rest included none whose V_{o_2} would have been markedly higher than the predicted value and the work load may be considered to have been only moderate in the entire series It follows that the slightly lower average value of the exercise factor in the older age group of the series may perhaps be considered a sign of slight abnormality of circulatory response to muscular work in comparison with conditions in the younger patients

Increase in SV on transition from rest to muscular exercise has been observed by many authors in normal subjects both in supine and in erect posture (e g Christensen 1931 Dexter et al 1951 Donald et al 1955 Musshoff et al 1959 Frick 1962 Levine et al 1962 Jernerus et al 1963) while some others could not elicit any such changes (e g Sjostrand 1956 Rushmer and Smith 1959 Bevegård et al 1960) The change in SV apparently depends largely on posture and on the level of external work (Chapman 1960 Braunwald et al 1967) Thus it increases in supine exercise on the average by 13 1 % on transition from rest to exercise by 16 % in old subjects and by 9 % in athlete it shows some slight further increase when the work load is increased and it changes more markedly at transition to work in the sitting position (Bevegård et al 1963 Granath and Strandell 1964 Ekelund and Holmgren 1967) In subjects at rest in recumbent position even the change from flat position to one in which the feet are raised to the pedals of the bicycle at the end of the table has been found to cause SVI to in crease by an average of 19 % from the initial value in healthy subjects followed by further rise up to 60 % during submaximal exercise (Frick and Somer 1964) According to Braunwald et al (1967) the primary response to exercise consists of simple tachycardia increased contractility due to sympathetic stimulation and of the Frank Starling mechanism all of which are essential at maximal exercise whereas only two of them need to operate during submaximal work or at rest Gorlin et al (1965) were able to demonstrate in healthy subjects three types of cardiac response to muscular exercise which manifest themselves in decreased unchanged or increased end-diastolic left ventricular volume In the first two types SV remained unchanged and the primary response was increase of the heart rate in addition to increased contractility In the third type the Frank-Starling mechanism too was apparently in operation and since as a rule the end-systolic ventricular volume diminished considerable increa es in SV were encountered The latter type of response might also be responsible for the considerable increase in SV noted in numerous cases during exercise in the present recatheterization series with the result of comparatively normal average relationship of Q and V_{o_2} during work despite the somewhat hypokinetic pattern at rest particularly in the young age group The changes in SV induced by exercise were similar in patients with and without pulmonary vascular involvement and they were not related to the changes in the $\bar{P}_{v}$ values which were observed to be elicited by change of posture or by exercise

The cardiac response to external work in terms of changes in $\dot{Q}$ and SV in relation to V_{o_2} is usually more of less abnormal in the presence of latent or manifest heart failure (e g Lewis et al 1953 Harvey et al 1962 Ross et al 1966) in that there is a small rise or no rise or a drop in SV and an abnormally small increase of Q over V_{o_2} As has been stated before no pattern of this kind was encountered in patients with elevated preoperative venous pressure and $\bar{P}_{RA}$ in the postoperative exercise tests of the present series in the entire series and in its subgroups even though the average left ventricular stroke work index was markedly higher compared to its value before surgery It seems hardly justified therefore to attribute the elevated venous pressure and $\bar{P}_{RA}$ in these cases to failing left ventricular function

Pressure and resistance findings

Right atrial pressures (P_{RA_a}, $\bar{P}_{RA}$ Table 20) — The right atrial *a* wave pressure (P_{RA_a}) averaged preoperatively in the entire recatheterization series 7 4 mm Hg and at recatheterization during rest 5 1 mm Hg The difference between the values before and after surgery is statistically significant in the entire series (***) in the group of patients younger than 40 years (***) and in the old age group (*) It increased significantly (***) during exercise to 8 9 mm Hg in the total series the corresponding rise was also statistically significant in the group of younger patients (**) but not in the old age group There were no significant differences between the sexes or the age groups P_{RA} was higher preoperatively, in the patients with pulmonary vascular disease (averaging 9 3 mm Hg) than in the others while it was on the level of the entire series after surgery

The right atrial mean pressure ($\bar{P}_{RA}$) was preoperatively 5.5 mm Hg on the average and 3 8 mm Hg at rest at follow-up The reduction is statistically significant in the entire series (***) and in both age groups (**) The pressure was slightly higher in the old than in the young age group both preoperatively and at rest postoperatively (** and * respectively) Its increase (***) during work brought it up to the average amount of 6 7 mm Hg about equally in both age groups There were only small differences between the sexes Raising of the feet upon the bicycle pedals prior to commencement of exercise caused a slight increase in $\bar{P}_{RA}$ (averaging 0 9 mm Hg in the 27 cases in which it was recorded) the increase was slightly greater in the old patients and the mean pressure further increased under the effect of work Elevated $\bar{P}_{RA}$ (over 5 mm Hg) was preoperatively present in 23 patients equally in both age groups and independent of involvement of pulmonary vascular disease Nine out of eleven patients with atrial fibrillation presented elevated $\bar{P}_{RA}$ in most instances up to 10 mm Hg or higher Postoperatively at rest $\bar{P}_{R}$ was elevated in 11 patients eight of them 40 years or older In four out of five cases with atrial fibrillation elevated $\bar{P}_{RA}$ was observed at rest in the follow-up examination Most of the patients with pulmonary vascular disease had normal $\bar{P}_{RA}$ at rest at follow up but most of them showed more or less abnormal increase of this pressure during exercise which was also noted in all patients with atrial fibrillation Altogether 35 patients had abnormally high $\bar{P}_{RA}$ during exercise, all those among them whose elevation amounted to 10 mm Hg or more were 40 years old or older

Right ventricular pressures (P_{RV_s} $P_{RV_{ed}}$), Table 20) — The systolic right ventricular pressure (P_{RV}) averaged preoperatively 42 2 mm Hg in the entire series and it was higher significantly (*) in the old than in the young age group The values were significantly lower on the average in the resting patients at follow-up (***), they had thus gone down to normal level in the entire series as well as in the young and old age groups (28 5 25 9 and 31 9 mm Hg respectively) The difference between the age groups was statistically significant (*) The P_{RV} during exercise averaged 53 9 mm Hg in the total series these values too were higher in the old age group than in the group of younger patients (***) There were no differences between sexes If the upper limit of normal variation is fixed at 35 mm Hg the value was within normal limits in 19 patients preoperatively, of whom only two belonged to the old age group At rest in the follow-up examinations abnormally high P_{RV} occurred in six patients only, five of them patients aged 40 years or older

The right ventricular end diastolic pressure ($P_{RV_{ed}}$) averaged preoperatively 6 4 mm Hg in the entire series it was slightly higher in the old than in the young age group (*) At follow-up at rest it was found to be lowered significantly (***) with normal average value 4 7 mm Hg and without any statistically significant differences between the age groups Its values during exercise averaged 7 4 mm Hg and they were higher in the old than in the young age group (***) If the upper limit of normal variation is fixed at 6 mm Hg the patients presenting elevated $P_{RV_{ed}}$ number 28 16 of them aged 40 years or older and including eight out of the 11 patients with atrial fibrillation and nine out of 11 with pulmonary hypertension and/or pulmonary vascular dis ease $P_{RV_{ed}}$ was still elevated at follow-up in ten patients about equally in both age groups and including four out of five cases with atrial fibrillation The value remained

at pre-exercise level or was lowered during work in 16 cases The other cases equally distributed among both age groups, mostly showed some slight increase but in 14 cases (equally distributed in the age groups) $P_{RV_{ed}}$ increased up to 10 mm Hg or higher

Pulmonary artery pressures (P_{PA_s} P_{PA_d} $\bar{P}_{PA}$ Table 20) — The systolic pulmonary artery pressure (P_{PA}) was preoperatively elevated on the average 38 0 mm Hg in the entire series the average of its values at rest at recatheterization was significantly lower (***) and normal 27 3 mm Hg while during exercise the average of 54 1 mm Hg was noted The respective values of the old age group were throughout significantly higher than those of the young age group (** ** ***)

The diastolic pressure (P_{PA_d}) averaged preoperatively 13 8 mm Hg at rest at follow-up 11 6 mm Hg and during exercise 22 7 mm Hg All values of the old age group were significantly superior to those of the young age group here too (* ** and ***) especially during exercise

The mean pulmonary artery pressue ($\bar{P}_{PA}$) averaged 22 6 mm Hg preoperatively 17 6 mm Hg at follow-up at rest and 33 8 mm Hg during exercise The $\bar{P}_{PA}$ value was higher in the old age group than in that of younger patients at rest both before and after surgery (* in both instances) and the average difference between the age groups during exercise amounted to 15 mm Hg (***)

Normal P_{PA} (30 mm Hg or less) was preoperatively recorded in 20 patients only three of them belonging to the old age group P_{PA} was higher than 50 mm Hg in six patients five of them aged 40 years or older At rest in the follow-up examinations it still exceeded 50 mm Hg in three of these six patients of whom one had a residual shunt the other three presented P_{PA} lower than 50 but in excess of 30 mm Hg The number of cases in the entire series with more than 30 mm Hg P_{PA} at rest at follow-up was 15 13 of them aged 40 years or older Elevated $\bar{P}_{PA}$ (in excess of 20 mm Hg) occurred at rest at follow-up in 12 patients one of whom had a residual shunt while the other 11 were patients aged 40 years or older $\bar{P}_{PA}$ was abnormally high during exercise at follow-up (higher than 30 mm Hg) in 23 patients 17 of them older ones

A systolic pressure gradient between the right ventricle and pulmonary artery was preoperatively observed in 34 patients of the entire recatheterization series in 12 patients at rest at follow-up and in 10 during exercise Its range was 0—21 mm Hg preoperatively 0—10 mm Hg at rest at follow up and 0—20 mm Hg during exercise

Pulmonary artery wedge pressure (P_w, $\bar{P}_w$ Table 21) — The *a* wave in the pulmonary artery wedge pressure (P_{w_a}) averaged in the entire recatheterization series 9.2 mm Hg preoperatively 9 0 mm Hg at rest at follow-up and 22 0 mm Hg during exercise which value is statistically significantly higher than that found at rest (***) No differences were noted between the sexes but the *a* wave during exercise had a higher amplitude in the group of patients aged 40 years or older than in the young age group (*)

The mean wedge pressure ($\bar{P}_w$) averaged preoperatively 8 0 mm Hg in the entire recatheterization series 7 6 mm Hg in its subgroup of younger patients and 8 5 mm Hg in that of older patients the corresponding values at rest at follow-up being 7 1 6 5 and 8 0 mm Hg respectively, with no statistically significant differences The male patients presented postoperatively a somewhat higher mean pressure at rest than the female patients (*) Elevation of the feet to the level of the bicycle pedals at the end of the table caused $\bar{P}_w$ to increase by 3 8 mm Hg on the average in the entire series the corresponding increase in the young age group was 2 4 mm Hg and in the old group it was 5 0 mm Hg the difference between the age groups being significant (**) Attendant on exercise whereby $\dot{Q}$ increased to 12 5 l/min on the average was a further increase in $\bar{P}_w$ $\bar{P}_w$ was significantly higher during work than at rest (***) it averaged 17 8 mm Hg in the entire series 14 7 mm Hg in the group of young patients whose $\dot{Q}$ averaged 13 8 l/min and 21 3 mm Hg in that of older patients with 11 0 l/min average $\dot{Q}$ the difference between the age groups was statistically significant (***)

The level of 14 mm Hg in $\bar{P}_w$ which may be considered to be the upper limit of its normal variation at rest was only exeeded in the case of two patients of the old age group at rest

Table 20 Pressures n r ght atr um (P_{R}) right ventricle (P_{R}) and pulmonary artery (P_{P}) in the group of patients subjected to recatheter zat on at follo v up — Symbols 1R 2R 2E and 2REΔ as in Table 18 2RR*Δ difference between values obtained at follow up at rest in flat position and with legs raised onto b cycle pedals

		P_{RA}								P_{R}						P_{PA}								
		a wave			Mean					Systolic			End diastolic			Systolic			Diastolic			Mean		
		1R	2R	2E	1R	2R	2E	2RR*Δ	2REΔ	1R	2R	2E	1R	2R	2E	1R	2R	2E	1R	2R	2E	1R	2R	2E
MALES	$\bar{x}$	8 1	5 7	7 0	5 9	4 8	5 3			47 1	32 2	55 8	6 6	5 5	7 2	42 2	30 8	51 9	16 5	13 9	25 0	26 8	20 4	34 4
	S D	1 9	1 8	1 8	2 4	?4	1 6			16 5	14 2	15 9	2 2	2 6	2 6	18 0	14 8	17 4	9 5	8 1	9 8	13 2	10 4	12 1
FEMALES	$\bar{x}$	7.2	4 9	9 6	5 4	3.3	7.3			40 0	27 0	53 1	6 3	4 3	7.5	36 1	25 9	51.2	12 5	10 7	21 8	20 8	18 4	33 5
	S D	3 2	2 1	3 6	3 0	1 8	4 0			12 3	7 7	?2 6	2 9	2 0	3 1	12 6	8 0	21 0	7.3	4 8	10 1	8 5	5 3	13 4
AGE	$\bar{x}$	6 8	4 6	10.2	4 6	3 2	5 9	0 8	2 7	38 6	25 9	42 4	5 7	4 4	6 3	33 5	24 2	40 0	11 7	9 6	18 5	20 1	15 6	27 1
	S D	2 7	1 9	3 4	?4	2 1	2 2	1 4	1 6	14.2	11 1	11.2	2 4	2 4	2 2	14 3	11.3	9 8	7 7	6.2	7 1	10 7	7 9	7 9
<40	r		0 213	0 125		0 325	0 262				0 471	0 ?22		0 287	0 301		0 523	0 161		0 431	0 251		0 522	0 245
	t		3 ? 8	3 804		3 016	3 699				5 4 0	6 541		2 470	3 680		4 123	6 428		1 598	6 044		2 737	6 592
	P(t)																			NS				
AGE	$\bar{x}$	8 7	5 8	8 2	6 7	4 5	7 3	1 1	4 0	46 8	31 9	68 4	7 3	5 1	8 9	43 9	31 4	66 3	16 5	14 3	?8 1	?6 0	20 1	42 1
	S D	3 3	2 0	3 3	2 9	1 9	4.3	1 4	3 0	12 5	8 1	21 2	2 8	2 0	3 1	12 8	8 2	20 0	8 0	5 0	10 8	9 2	5 7	13 2
≥40	r		−0 059	−0 217		0 066	0 385				0 850	0 674		0 185	0 381		0 78	0 705		0 559	0 700		0 672	0 717
	t		2 682	1 955		3 081	2 435				10 564	10 319		2 796	5 890		7 664	10 803		1 653	8 078		4 319	10 793
	P(t)			NS																NS				
ALL	$\bar{x}$	7 4	5 1	8 9	5 5	3 8	6 7	0 9	3.2	42 2	?8 5	53 9	6 4	4 7	7 4	38 0	27 3	51 4	13 8	11 6	22 7	22 6	17 6	33 8
	S D	3 0	2 0	3 4	2 8	2 1	3 6	1 4	2 4	14 0	10 3	20 8	2 7	2 2	2 9	14 6	10 6	19 9	8 2	6 1	10 0	10 4	7 3	12 9
	r		0 193	−0 104		0 847	0.291				0 645	0 540		0 106	0.368		0 676	0 565		0 559	0 575		0 627	0 558
	t		4 516	4 111		4 200	3 851				9 675	10 429		3 741	6 489		7 553	10 665		2 401	9 876		4 675	11 120
	P(t)																							
M/F	t	1 044	1 312	1 891	0 666	2 363	1 637			1 633	1 4?4	0 488	0 434	1 576	0 341	1 301	1 ?88	0 1?4	1 588	1 516	1 204	1 770	1 505	0 242
	P(t)	NS	NS	NS	NS		NS			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	AS	AS
<40/~40	t	1 751	2 086	1 152	2 922	2 447	0 971	0 553	1 883	2 332	2.358	5 322	2.293	1 075	3 347	2 893	2 786	5 796	2 300	3 168	3 695	2.254	2 493	4 902
	P(t)	NS	NS	NS			NS	NS	NS					NS										

Table 21 Pressures in pulmonary artery wedge position (P_W) left atrium (P_{LA}), left ventricle (P_{LV}) and systemic artery (P) in the group of patients subjected to recatheterization at follow-up Symbols 1R 2R 2E and 2REΔ as in Table 18 and 2RR*Δ as in Table 20

		P_W								P_{LA}		P_{LV}		P								
		a wave			Mean					*a* wave	Mean	Syst	End Diast	Systolic			Diastolic			Mean		
		1R	2R	2E	1R	2R	2E	2RR Δ	2REΔ	1R	1R	1R	1R	1R	2R	2E	1R	2R	2E	1R	2R	2E
MALES	$\bar{x}$	12 0	10 8	20 9	9 2	8 2	16 8	2 2	9 4	9 3	5 8	132 6	7 2	132 2	152 9	200 8	84 4	84 4	95 8	10? 7	107 8	125 8
	S D	4 4	3 9	7 4	2 8	2 3	5 8	2 9	5 3	2 9	.5	24 4	2 8	18 5	23 5	41 6	9 3	12 8	17 0	13 3	14 7	21 6
FEMALES	$\bar{x}$	8 8	8 3	22 4	7 4	6 6	18 1	4 0	11 4	10 2	6 8	134 0	8 0	133 2	143 3	190 7	79 5	74 7	85 8	99 4	98.2	122 3
	S D	3 0	3 8	11 0	2 7	3 4	8 0	2 6	7 2	3 9	2 8	24 8	2 8	22 5	22 8	31 7	12 0	12 2	11 2	14 7	14 9	19 0
AGE <40	$\bar{x}$	9 2	8 2	17 9	7 6	6 5	14 7	2 4	8 5	8 8	5 4	120 4	6 8	123 8	137 6	185 8	76 8	75 4	86 5	94 6	95.3	117.3
	S D	3 5	3 6	7 8	2 8	3 0	6 7	2 4	6 0	3 1	2 1	16 4	2 4	14 1	18 0	31 2	10 0	14 0	13 5	12.3	14 2	16 4
	r	0 139	0 327		0 .00	0 369								0 052	0 7?2		0 485	0 692		0 505	0 679	
	t	0 942	5 257		1 558	6 058								3 524	11 902		0 628	5 726		0 300	9 781	
	P(t)	NS			NS	*											NS			NS		
AGE ≧40	$\bar{x}$	9 2	10 2	?6 7	8 5	8 0	21 3	5 0	13 8	12 1	7 8	148 8	8 8	144 9	157 1	203 6	86 7	80 8	91 4	108 1	108.5	131 0
	S D	3 0	4 3	10 8	2 8	3 3	6 6	2.3	6 5	3 9	2 9	23 1	2 9	23 1	24 8	37 0	10 8	11 4	13 8	13.2	13 7	20 7
	r	0 201	0 334		0 199	0 3 9								0 438	0 554		0 2?3	0 623		0 340	0 544	
	t	0 532	6 060		0 592	10 287								2 398	7 199		2 131	4 603		0 129	6 298	
	P(t)	NS			NS															NS	*	
ALL	$\bar{x}$	9 2	9 0	22 0	8 0	7 1	17 8	3 8	10 8	9 8	6 9	132 7	7.2	132 9	146 2	193 5	81 1	77 7	88 6	100 4	101 1	123 4
	S D	3 3	4 0	10 2	2 8	3 2	7 4	2 7	6 7	3 7	2 8	21 ?	2 8	21 2	23 2	34 7	11 4	13 1	13 7	14 3	15 4	19.5
	r	0 016	0 363		0 227	0 421								0 436	0 665		0 420	0 676		0 541	0 668	
	t	0 10	7 400		1 674	11 495								4 283	13 283		1 941	7 456		0 372	11 191	
	P(t)	NS			NS												NS			NS		
M/F	*t*	1 214	1 8?3	0 415	1 892	2 053	0 652			0 612	1 057	0 138	0 660	0 178	1 424	0 848	1 600	2 763	2 105	0 846	2 ?47	0 563
	P(t)	NS	NS	NS	NS		NS			NS	NS	NS	NS	NS	NS	NS	—			NS		NS
<40/≥40	*t*	0 000	1 536	2 5?6	1 0?2	1 ?50	3 570	2 927	3 080	2 198	2 947	3 559	1 820	4 033	3 309	1 656	3 569	1 605	1 293	3 972	3 552	2 645
	P(t)	NS	NS		NS	NS							NS			NS		NS	NS			

in the follow-up examinations The exercise factor of one of these was normal and that of the other who had atrial fibrillation was slightly reduced the SV of both during work was nearly unchanged Both had mild arterial hypertension and slightly elevated $\overline{P}_{RA}$ preoperatively and at rest after surgery The level of 20 mm Hg was exceeded during exercise only in three of the younger patients all of them having normal exercise factor and presenting distinct increase of SV during exercise and in two patients of the older group who developed $\overline{P}_{W}$ higher than 30 mm Hg both had a normal exercise factor but the SV of one of them did not change and that of the other decreased during exercise Arterial hypertension was not present in any of these five patients

Left atrial pressures (P_{LA} $\overline{P}_{LA}$ Table 21) — The left atrial pressures were recorded preoperatively in 26 instances in the present series of recatheterized patients The *a* wave amplitude (P_{LA}) averaged 9 8 mm Hg in the entire series 8 8 mm Hg in the younger patients and 12 1 mm Hg in the group of older patients The corresponding mean pressures were 6 9 5 4 and 7 8 mm Hg The *a/m* ratio averaged 1 64 in the younger and 1 55 in the older age group P_{LA} as well as $\overline{P}_{LA}$ were significantly higher in the old age group than in the group of younger patients at a statistically significant level (* and ** respectively)

Left ventricular pressures (P_{LV} $P_{LV_{ed}}$ Table 21) — The left ventricular pressure was preoperatively recorded in 24 instances of the recatheterization series The systolic pressure (P_{LV}) averaged 132 7 mm Hg in the entire series 120 4 mm Hg in the group of younger patients and 148 8 mm Hg in that of older patients The difference between groups was statistically significant (**) The mean of the end-diastolic pressures $P_{LV_{ed}}$ was on the average 7.2 mm Hg in the total series 6 8 mm Hg in the young and 8 8 mm Hg in the old age group without any statistically significant difference The highest $P_{LV_{ed}}$ encountered was 13 mm Hg which is still within normal range

Systemic arterial pressures P_{s} P_{d} $\overline{P}$ Table 21) — The systolic arterial pressure (P) averaged in the entire series 132 9 mm Hg preoperatively 146 2 mm Hg at rest at follow-up, and 193 5 mm Hg during exercise at follow-up both latter values representing a statistically significant change (***) This pressure was markedly higher at rest in the old age group than in the group of younger patients both preoperatively and at follow up (*** and ** respectively) but not during exercise in the tests after the operation

The diastolic arterial pressure (P_{d}) averaged in the entire series 81 1 mm Hg preoperatively 77 7 mm Hg at rest after surgery and 88 6 mm Hg during exercise at follow-up it was significantly higher in the old age group than in the younger group at statistically significant level (***) before but not after surgery

The mean arterial pressure (P_{a}) averaged preoperatively 100 4 mm Hg in the entire series 101 1 mm Hg (i e nearly equal) at rest at follow-up and 123 4 mm Hg during exercise which represents a statistically significant increase (***) from the value at rest The $\overline{P}$ in the old age group was on the average significantly higher than in the younger group preoperatively and at follow-up during rest and exercise (*** *** and * respectively) The male patients presented postoperatively slightly higher P_{a_d} and P than the female patients

P_{d} of 100 mm Hg or higher was preoperatively encountered in five patients four of them aged 40 years or older, and in five patients at follow-up of whom three belonged to the older subjects The (P_{d} level of 100 mm Hg was reached or exceeded during exercise by 15 patients eight of them belonging to the older age group

Pulmonary and systemic vascular resistance (R_{p} R_{p_i} R R_{i} R_{p}/R Table 22) — The pulmonary vascular resistance index (R_{p_i}) averaged preoperatively 185 4 dynes sec cm^{-5} in the entire series it was slightly higher though not significantly in the old than in the young age group Both in the entire series and in its subgroups the average values were within normal range (not in excess of 250 dynes sec cm^{-5}) Abnormally high R_{p_i} values were preoperatively found in 12 cases ten of them patients aged 40 years or older Three of the 12 patients presented a value higher than 500 dynes sec cm^{-5} The average value of R_{p}

at rest at follow up was 265 7 dynes sec cm^{-5} in the total group the mean in the old age group was significantly higher than that in the younger group (**) The increase from the preoperative value was statistically significant in the total series and in the old age group (*** and ***) but not in the young age group There was no difference between sexes Exercise caused statistically significant reduction of R_p both in the entire group and in the young and old age groups (*** *** and * respectively) resulting in the average values of 189 8 119 5 and 281 5 dynes sec cm^{-5} Also during exercise the older age group presented higher values of the index than the younger group (***) Of the 12 out of 14 cases with preoperative pulmonary vascular involvement in which complete postoperative data existed six still presented elevated P_{PA} at rest in the follow-up examinations and in seven it was present during exercise One of the patients had residual left to right and right-to-left shunts and pulmonary hypertension while in one patient no pulmonary pressure record was obtained but elevated pressure was likely on clinical grounds The preoparetively elevated R_p showed equally common reduction of increase after surgery independent of the severity of the vascular disease

The systemic vascular resistance index *(R)* averaged preoperatively in the entire recatheterized series 3003 7 dynes sec cm^{-5} the value in the group of patients aged 40 years or older was markedly higher than that of the younger group (***) The postoperative index of the entire group 2378 2 dynes sec cm^{-5} was significantly lower than the preoperative value (***) and the same is true for the values in the young and old age groups (*** and ** respectively) There were no differences between the sexes whereas here too the values of the older patients were clearly higher than those in the younger group (***)

Table 22 Pulmonary vascular resistance index (R_p) systemic vascular resistance index *(R)* and ratio of pulmonary and systemic resistances (R_p/R) in the group of patients subjected to recatheterization at follow-up — Symbols 1R 2R and 2E as in Table 18

		R_p			R			R_p/R		
		1R	2R	2E	1R	2R	2E	1R	2R	2E
MALES	$\bar{x}$	217.2	289 7	199 5	3044 7	2378 0	1345 7	0 075	0 115	0 149
	S D	249 7	260 4	147 4	7 8.3	719 5	374.3	0 090	0 079	0 082
FEMALES	$\bar{x}$	171 1	255 5	186 0	2985 2	2378 1	1336 1	0 058	0 103	0 135
	S D	160.2	134 0	141 0	937 8	683 9	443 6	0 045	0 040	0 082
AGE	$\bar{x}$	144 7	197 1	119.5	2649.3	2045 8	1109 9	0 058	0 096	0 113
	S D	164 7	121 7	55 9	656.3	480 9	235 1	0 066	0 057	0 053
<40	r		0 234	0 281		0.285	0 4 9		0 326	0.245
	t		1 660	3 684		4 970	12 145		2.970	1.350
	P(t)		NS							NS
AGE	$\bar{x}$	239 1	353 6	281.5	3471 5	2803 3	1637 4	0 070	0 120	0 172
	S D	213.3	203 8	166 4	912 0	686.3	4[illegible]7 4	0 057	0 048	0 097
≧40	r		0 697	0 636		0.286	0 490		0 616	0 745
	t		3 521	2.208		3 437	9.585		5.314	3 622
	P(t)									
ALL	$\bar{x}$	185 4	265 7	189 8	3003 7	2378 1	1338 8	0 063	0 106	0 139
	S D	191.3	179.2	141 5	872.2	688.2	4[illegible]1 6	0 062	0 054	0 082
	r		0 588	0 685		0 467	0 654		0 455	0.538
	t		3 616	4.317		5 790	15 067		5.328	3.396
	P(t)									
M/F	t	0 719	0 513	0.304	0.262	0 000	0 080	0 745	0 6[illegible]4	0 578
	P(t)	NS	NS	NS	NS	NS	NS	NS	NS	NS
<40/≧40	t	1.837	3 400	4 479	3.820	4 692	5.333	0 694	1 701	2 601
	P(t)	NS						NS	NS	

R was strongly lowered during exercise (*** in the entire series and in both subgroups) it averaged then 1338 8 dynes sec cm^{-5} in the entire series

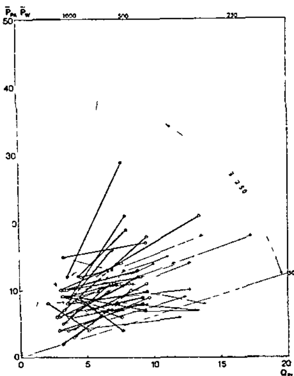
In order to reveal any relative changes of resistances which might occur the ratio of the pulmonary and systemic vascular resistances (R_p/R) was determined in each instance This ratio averaged preoperatively in the total series 0 063 the average at rest in the follow-up examination 0 106 is significantly higher (***) and during exercise a further rise (**) up to 0 139 on the average took place The values in the old age group differed from those of the younger group significantly (*) only during exercise when they were higher than the latter No differences were established between sexes

The gradients between mean pulmonary artery pressure and mean wedge pressure ($\bar{P}_{PA}—\bar{P}_{W}$) were plotted against Q_p (Figs 19 a and b) in order to illustrate the relationships of blood flow pressure gradient and resistance in the pulmonary circulation As can be seen the changes in flow-pressure relationship mostly occurred approximately along the isoresistance lines However a general trend towards higher resistance level following operation can be discerned particularly in the old age group in some cases (likewise more commonly among the older patients) in fact a marked shift towards higher resistance level ensued on the operation The gradients between mean systemic arterial pressure and mean right atrial pressure ($\bar{P}$ —$\bar{P}_{RA}$) have been similarly plotted against Q in Figs 20 a and b The trend revealed by these graphs is slight lowering of R_{i} from the preoperative values to those at rest after surgery and marked lowering of R during exercise in nearly identical manner in both age groups merely with somewhat less powerful reduction observable in the group of older patients

Comments on the pressure findings — The operation resulted in normalization of pressure relations in the central circulation in the majority of cases both *in the group of patients* younger than 40 years and in the old age group This is consistent with the findings already reported by Blount et al (1953 1954) However, the average values were generally higher throughout in the old than in the young age group At follow-up elevated pressures were more commonly encountered also in patients with atrial fibrillation and in those with pulmonary vascular disease

The left and right atrial pressures at rest (the left atrial pressure having been estimated from the pulmonary artery wedge pressure under assumption of an approximate 1 1 relation see page p 90) often displayed inverse behaviour in respect of their change from before to after surgery The mean values in the left atrium did not change significantly however The right atrial pressures were preoperatively abnormally high on the average but were significantly reduced as a result of the operation in contradiction of Arnfreds (1967 a) observations but in agreement with the findings of Petersson (1967) In seven cases of ASD secundum (four younger and three older patients not included in the present follow-up series) a small Teflon catheter was left in connection with the operation in the right atrium as well as transseptally in the left atrium and the atrial pressures were followed during the first two postoperative days No systematic behaviour of the interatrial pressure gradient was observed during this period apart from earlier rise of the left atrial pressure when the blood volume was expanded by rapid blood transfusion Similar observations have been reported by Rehder et al (1962) who also noted that the spontaneous changes occurring in the atrial pressures in the immediate postoperative period were the inverse of those seen in the cardiac output No significant abnormal elevation of the left atrial pressures was observed immediately after closure of the atrial septal defect nor were any established at follow-up in the present series

The filling pressure of the right ventricle was significantly lowered as a result of the operation The left ventricular filling pressure which was slightly low before surgery was not directly measured after the operation and for this reason $\bar{P}_{RA}$ and $\bar{P}_{W}$ were substituted as measures of the right and left ventricular filling pressures assuming an approximate 1 1 relationship between these pressures and $P_{RV_{ed}}$ or $P_{LV_{ed}}$ respectively (see p 90) Elevation of the atrial mean pressures past the ventricular filling pressures as a result of potential superposition of the atrial and ventricular contrac



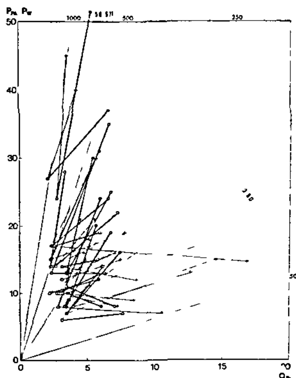
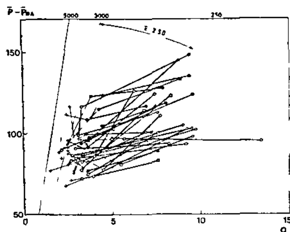


Fig 19 Gradients between pulmonary artery mean pressure and mean wedge pressure before operation at rest and after operation at rest and during exercise plotted over pulmonary flow index in patients of the young (Fig 19 a) and old (Fig 19 b) age group The thin lines are isoresistance lines (scale in dynes sec cm^{-5} in the margin) with the sector consistent with normal mean $\pm$ 2 S.D indicated by an arc with arrow tips + Male or female patients before operation ● Male after operation o Female after operation



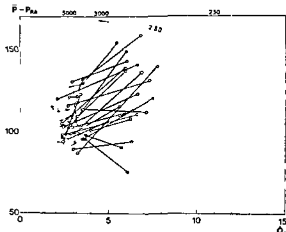


Fig 20 Gradients between arterial mean pressure and right atrial mean pressure before operation at rest and after operation at rest and during exercise plotted over the systemic flow index in patients of the young (Fig 20 a) and old (Fig 20 b) age group — The thin lines are isoresistance lines (scale in dynes sec cm^{-5} in the margin) with the sector consistent with normal mean $\pm$ 2 S.D indicated by an arc with arrow tips + Male or female patients before operation ● Male after operation o Female after operation

tions at high heart rates (Sarnoff and Mitchell 1961) was ruled out by the observation that owing to the moderate external work load also the heart rate was moderate in the cases with sinus rhythm even during exercise and that the P—R times were in all cases shorter than the corresponding diastolic filling periods, in spite of elevated heart rate

Expressed in terms of $\bar{P}_{RA}$ the right ventricular filling pressure was lower at follow-up than preoperatively in both age groups the average SVI_{RV} correspondingly amounted to about one third and the average P_{RV} to about two thirds of the preoperative value while the outflow resistance was approximately 50% higher than the preoperative average The filling pressure of the right ventricle increased once more during exercise at follow up while simultaneously SVI_{RV} increased by about one fourth P_{RV_s} was almost doubled and the outflow resistance was lowered approximately to preoperative level on the average The normal behaviour for the right ventricular filling pressure in young individuals is to remain at resting level or to decrease during exercise whereas in old healthy subjects it usually increases in positive correlation with the changes in $\bar{P}_{PA}$ and $\bar{P}_{w}$ (Bevegard et al 1963 Granath and Strandell 1964) Likewise the right ventricular filling pressure increases during physical work in cases presenting abnormal increase in $\bar{P}_{PA}$ during exercise as a result of left ventricular failure or in which right ventricular failure is present (Harvey et al 1962) Since the changes in SVI parallelled the changes in filling pressure and since the increase in Q relative to the augmentation of V_{O_2} during exercise was normal in the majority of the cases it seems that the rise of the average ventricular filling pressure may not be considered any sign of abnormal of failing right ventricular function it would rather be a normal response to acute increase of volume and pressure load in accordance with Frank Starling s law In eleven cases most of them older patients the filling pressure was slightly elevated at rest at follow up perhaps a sign of slightly abnormal right ventricular function Exercise was attended by more pronounced elevation of the filling pressure in only one of these in which it rose to 18 mm Hg but the blood flow response was normal and P_{PA} was elevated up to 73 mm Hg

In terms of $\bar{P}_{LA}$ as well as $\bar{P}_{w}$ the left ventricular filling pressure was preoperatively normal or slightly low in the entire series and in its subgroups and it did not change on the average as a result of surgery $\bar{P}_{w}$ at follow up at rest and during exercise has been plotted against $\dot{Q}$ and against SVI in Fig 21 It is seen that the increases in left ventricular filling pressure during work related to the change in Q_i were higher in the old than in the young age group Moreover, larger increase of the left ventricular filling pressure during work was usually associated with increase of SVI in the younger group while in the older group this relationship was less pronounced and more frequently absent In both age groups the increase in $\bar{P}_{w}$ during work was slightly greater on the average in relation to Q than in healthy individuals (Ekelund and Holmgren 1967) although the response may be considered to range within normal limits in the majority of the patients $\bar{P}$ and R were high within normal range at rest after surgery in both age groups but somewhat high values were seen at exercise particularly in the old age group as compared to findings made in healthy, sedentary individuals of various ages (Grimby 1962 Granath et al 1964 Ekelund and Holmgren 1967 Julius et al 1967) As was demonstrated by the radiological findings there was evidence of smaller cross-sectional area of the aortic arch in patients with ASD which was more pronounced in older individuals and the slight increase seen at follow-up in this cross sectional area was less in the older patients One may accordingly presume that the total outflow impedance of the left ventricle would be enhanced in the present series as compared to healthy individuals particularly in older patients whose aortic walls are likely to be more rigid The altered viscoelastic properties of the left ventricular outflow system have been argued to account also for the similar relationship between $\bar{P}_{w}$ and $\dot{Q}$ during exercise in healthy old individuals (Granath et al 1964) As has been mentioned before abnormal elevation of the left ventricular filling pressure during exercise was encountered in five cases with normal or almost normal blood flow response in all but one case in which SVI was reduced during exercise In six patients

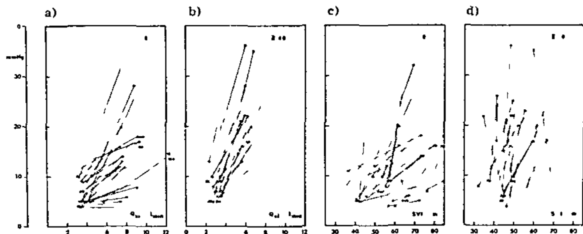


Fig 21 Pulmonary artery wedge pressure found at rest and during exercise at follow up plotted over systemic flow index (Fig 21 a Young age group Fig 21 b Old age group) and over stroke volume index (Fig 21 c Young age group Fig 21 d Old age group)

with abnormally low exercise factor only one of them a patient younger than 40 years $\bar{P}_w$ during exercise was normal ranging from 9 to 26 mm Hg (average 11 7 mm Hg) *SV* was lowered in all these during exercise in greater or lesser degree In nine patients who displayed reduction of *SV* during exercise including four younger patients $\bar{P}_w$ was normal in all but one ranging from 4 to 22 mm Hg (average 11 7 mm Hg) while it was 35 mm Hg in one female subject This latter case presented 8 mm Hg preoperative $\bar{P}_{LA}$ No apparent cause was discovered for this patient s left ventricular abnormality but she had clinically slight symptoms and signs of left ventricular failure and was relieved of the symptoms on digitalization In almost all cases thus the response of the left ventricular filling pressure to exercise was within normal range although it is true that high normal values were recorded during exercise in most instances Similarly as for the right ventricle the increased left ventricular filling pressure during exercise can be regarded to be a normal response to suddenly increased volume and pressure load abnormally high respon e was encountered in a few cases in only one of which left ventricular failure was apparently present In the series of uncomplicated ASD reported by Petersson (1967) the filling pressures showed much lesser changes in response to exercise in adults the average right ventricular filling pressure being reduced and the average wedge pres ure increasing by only 3—4 mm Hg

Raising of the legs up to the level of the bicycle pedals elicited in numerous patients elevation both of $\bar{P}_{RA}$ and particularly of $\bar{P}_w$ which was often superior to the subsequent increase due to the work proper Similar observations have been reported by other authors in healthy subjects too (Granath et al 1964) This change in posture has also been shown to induce in healthy subjects significant increase of *Q* (Frick and Somer 1964) and likewise of the total cardiopulmonary blood volume (Levin son et al 1966) The pulmonary vascular bed is normally characterized by great distensibility for which reason shift of large blood volumes into the pulmonary circulation is only associated with small changes in pulmonary distending pressure P_D (Yu et al 1967 $P_D = (\bar{P}_{PA} + \bar{P}_{LA}) / 2$) In the present series the elevation of the legs sometimes produced an increase amounting to as much as 5 mm Hg in P_D (as calculated from the mean pulmonary artery and mean wedge pressures) which would imply either transfer of a large blood volume from the periphery to the lungs having high vascular distensibility or a smaller one in combination with reduced pulmonary vascular distensibility The changes in $\bar{P}_{RA}$ $\bar{P}_w$ and P_D had no relation to presence or absence of pulmonary hypertension to radiological signs of pulmonary venous congestion to the heart volume preoperative magnitude of the shunt age or lung function findings

R_p which is low in uncomplicated ASD usually displays slight rise after surgery (e g

Petersson 1967) In cases with complicating pulmonary vascular disease it is often reduced postoperatively (Blount 1953 Beck et al 1960) but many reports indicate great irregularity of this reduction (Besterman 1961, Braunwald and Braunwald 1962 Reeve et al 1966) In fact serial postoperative catheterizations (Reeve et al 1966 Craig and Selzer 1968) have demonstrated that the pulmonary vascular disease is often irreversible and one may also encounter cases in which it progresses postoperatively Pulmonary hypertension and/or pulmonary vascular disease was preoperatively present in 14 patients of the present recatheterized series In two of them the condition was classified as hyperkinetic pulmonary hypertension (HPH P_{PA} 50 mm Hg or higher R_{p_1} normal i e not above 250 dynes sec cm^{-5}) in three others as obstructive pulmonary hypertension (OPH R_p higher than 500 dynes sec cm^{-5}) and in nine cases it was classified as incipient pulmonary vascular disease (IPVD R_p ranging between 251 and 500 dynes sec cm^{-5}) Complete postoperative data were available in 12 of these cases One case with OPH presented reduced R_{p_1} postoperatively in the other R_p was increased In both cases with HPH increased R_p was found as well as in half of the cases with IPVD In the patient presenting residual shunt and pulmonary hypertension at follow-up R_{p_1} had been doubled from its preoperative value from 362 to 760 dynes sec cm^{-5} This patient had two weeks after surgery an attack of dyspnoea right thoracic pain cyanosis and slight haemoptysis associated with enhanced right ventriqular thrust and increased loudness of the pulmonary second sound and with disappearance of the distal portion of the shadow of the right lower lobe branch of the pulmonary artery in the chest radiogram This complication was considered to consist of pulmonary artery embolism or thrombosis It was rapidly followed by signs suggesting presence of a shunt and it is likely that the recurrence of the shunt at this stage saved the patient from pulmonary infarction and severe deterioration or death (Canada et al 1953) Advanced obstructive changes including atheromatosis and thrombosis dilated pulmonary arteries and low blood flow rate in the pulmonary circulation have been considered to be factors predisposing to pulmonary artery thrombosis in ASD with obstructive pulmonary hypertension (e g Dexter 1956) The same conditions are present after closure of the defect in particular in old subjects with more advanced vascular changes and with hypokinetic pulmonary circulation 13 out of 18 patients presenting increase of the pulmonary vascular resistance index up to ab normally high value after surgery were older patients The cause responsible for the preponderance of older patients is obscure but potential occurrence of postoperative throm botic processes in the pulmonary arterial tree is a possibility deserving attentation

Derived parameters of systolic and diastolic ventricular functions

Stroke work index (SWI Table 23) — Equal values of the average right ventricular *stroke work index (SWI_{RV}) were found in both* age groups of the present series and in both sexes preoperatively as well as after surgery It was reduced by more than two-thirds (***) on operation and during exercise at follow-up it was approximately double (***) compared to the values at rest The left ventricular stroke work index *(SWI_{LV})* averaged in the entire series 53 8 g m preoperatively 79 5 g m at rest after surgery, and 105 4 g m during exercise at follow-up An average increase by 47 7 % of SWI_{LV} thus ensued on operation in the total series the corresponding percentage being 45 0 and 50 8 % in the young and old age group, respectively The increase of this index during exercise averaged 36 4 % in all cases the value of the index was lower (**) and its change smaller (*) in the old than in the young age group The changes seen in individual cases during work were usually consistent with the changes of the stroke volume index in spite of the elevated arterial pressure level during exercise SWI_{LV} thus decreased in eight patients during exercise six of them old subjects

Pressure time index (PTI Table 23) — The pressure-time index per beat for the right ventricle *(PTI_{RV})* averaged in the entire recatheterized series 11 5 mm Hg sec preoperatively 8 4 mm Hg sec at rest at follow-up and 12 4 mm Hg sec during exercise at follow-up the means representing statistically significant changes (*** in both instances) the values in the group of older patients were distinctly higher than those of the young age group preoperatively as well as at follow-up

at rest and during exercise (** *** and *** respectively) There were no differences between sexes 14 patients presented PTI_{RV} in excess of 10 0 mm Hg sec which is not usually exceeded in normal cases seven of them belonged to the group with pulmonary vascular involvement and six of them had atrial fibrillation

The pressure-time index per beat for the left ventricle (PTI_{LV}) averaged in the entire recatheterized series 35 1 mm Hg sec preoperatively 41 6 mm Hg sec at rest at follow-up implying statistically significant increase (***) and 39 2 mm Hg sec at follow-up during exercise i e slight lowering from the values at rest (*) There were no sex differences but the values were higher in the young than in the old age group preoperatively as well as at rest postoperatively (** in both instances) but not during exercise Five patients whose arterial diastolic pressure was elevated past 100 mm Hg had PTI_{LV} indices averaging 50 3 mm Hg sec The value of PTI_{LV} of 46 0 mm Hg sec which is not often normally exceeded was passed by four patients before the operation and by 12 patients at rest after surgery ten of whom were aged 40 years or older

Mean systolic ejection rate (MSER Table 23) — The right ventricular mean systolic ejection rate ($MSER_{RV}$) averaged preoperatively in the total series 345 7 ml sec^{-1} and was lowered by the operation to 119 8 ml sec^{-} (***) during exercise it increased by 62 2 % on the average (***) The values were higher throughout in the old than in the young age group particularly those referring to exercise (***) There were no differences between sexes

The left ventricular mean systolic ejection rate ($MSER_{LV}$) averaged preoperatively in the entire series 120 1 ml sec^{-} the values in the old age group were significantly lower than those in the younger group (**) The values increased significantly (***) after surgery averaging 149 6 ml sec^{-} in the total series while the values of the patients younger than 40 years were higher than those of the older subjects (***) Further increase (***) was caused by exercise amounting to 55 5 % in the entire series 59 0 % in the young and 51 0 % in the old age group The values were higher in the young than in the old age group preoperatively as well as at rest and during exercise at follow-up (** *** and *** respectively) There were no sex differences Increase of $MSER_{LV}$ and $MSER_{RV}$ in lesser amount than 15 % or their reduction was seen in three older and two younger patients during work all these also presented simultaneous reduction of the stroke volume index and stroke work index

Parameters of ventricular diastolic function (Table 24) — The ventricular distensibility characteristics were approached by calculating three different indices *D-1 D-2* and *D-3* for each ventricle

In the *right ventricle* $D\text{-}1_{RV}$ averaged in the entire recatheterized series preoperatively 22 4 ml mm Hg^{-1} it was significantly less 14 2 mm Hg^{-1} at rest at follow-up (***) and even lower 9 2 ml mm Hg^{-1} during exercise (***) The older patients presented markedly lower values than the younger ones both preoperatively and at rest and during exercise at follow-up (** * and *** respectively) There were no sex differences The $D\ 2_{RV}$ index averaged preoperatively in the entire series 32 5 ml mm Hg^{-1} postoperatively at rest it was much lower 12 8 ml mm Hg^{-} (***) Its values in the old age group were lower than those in the younger group both preoperatively and at follow-up (* and **) No sex differences were established The $D\text{-}3_{RV}$ index averaged in the entire series 1 5 preoperatively and 1 1 after surgery indicating a statistically significant change (**)

In the *left ventricle* $D\text{-}1_{LV}$ averaged in the total recatheterized series 6 0 ml mm Hg^{-1} preoperatively at rest at follow-up it was significantly more 8 5 ml mm Hg^{-} (**) and it was lowered to 4 3 on the average during exercise (***) The values were lower in the old than in the young age group preoperatively and postoperatively at rest and during exercise (* ** and *** respectively) they were also lower for males than females preoperatively and at rest after surgery (* in both instances) The $D\text{-}2_{LV}$ index averaged in all cases 9 0 ml mm Hg^{-1} preoperatively and it was higher 12 7 ml mm Hg^{-1} (**) at rest in the follow-up examinations The values of the older patients were significantly higher (*) than those in the young age group before but not after surgery no sex differences were observed The $D\ 3_{LV}$ index presented the same average 1 6 preoperatively and postoper-

Table 23 Stroke work index of right ventricle (SWI_{RV}) and left ventricle (SWI_{LV}) pressure-time index of right ventricle (PTI_{RV}) and left ventricle (PTI_{LV}) and effective mean systolic ejection rate of right ventricle ($MSER_{RV}$) and left ventricle ($MSER_{LV}$) in the group of patients subjected to recatheterization at follow-up — Symbols 1R 2R 2E and 2RE∆ as in Table 18

		SWI_{RV} 1R	SWI_{RV} 2R	SWI_{RV} 2E	SWI_{RV} 2RE∆ %	SWI_{LV} 1R	SWI_{LV} 2R	SWI_{LV} 2E	SWI_{LV} 2RE∆ %	PTI_{LV} 1R	PTI_{LV} 2R	PTI_{LV} 2E
MALES	$\bar{x}$	44.9	12.7	29.5	140.3	52.2	85.4	108.7	34.1	11.2	8.4	12.5
	SD	13.6	3.3	8.7	118.3	17.7	20.7	32.1	47.4	3.0	2.4	3.4
FEMALES	$\bar{x}$	43.0	12.0	27.9	176.6	54.5	77.1	104.0	37.3	11.7	8.4	12.3
	SD	21.4	4.5	11.1	74.4	16.0	22.3	24.1	31.4	3.7	2.9	5.5
AGE < 40	$\bar{x}$	43.5	11.6	26.0	132.0	55.2	80.3	113.7	48.8	10.2	7.3	9.9
	SD	23.4	3.6	8.4	93.5	18.0	18.4	23.6	37.2	2.8	1.9	3.1
	r		0.361	0.788			0.418	0.355			0.374	0.473
	t		7.950	9.392			7.055	7.462			5.686	4.992
	P(t)											
AGE ≥40	$\bar{x}$	43.6	12.9	31.2	129.0	52.1	78.6	94.8	23.3	13.2	9.8	15.4
	SD	16.7	4.7	12.0	84.1	14.2	26.1	26.5	31.0	3.7	2.9	5.1
	r		0.004	0.668			0.287	0.472			0.692	0.715
	t		8.857	9.148			5.118	2.931			6.314	7.321
	P(t)											
ALL	$\bar{x}$	43.6	12.2	28.4	130.7	53.8	79.5	105.4	36.0	11.5	8.4	12.4
	SD	20.5	4.2	10.4	88.5	16.4	22.0	26.4	36.2	3.5	2.7	4.9
	r		0.198	0.541			0.342	0.397			0.639	0.733
	t		11.698	13.016			8.494	7.028			8.482	8.217
	P(t)						*					*
M/F	t	0.330	0.639	0.550	0.418	0.451	1.30	0.512	0.241	0.526	0.000	0.153
	P(t)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<40/≥40	t	0.019	1.133	1.755	0.170	0.720	0.274	2.680	2.484	3.353	3.700	4.530
	P(t)	NS	NS	NS	NS	NS	NS					

		PTI_{RV} 1R	PTI_{RV} 2R	PTI_{RV} 2E	$MSER_{RV}$ 1R	$MSER_{RV}$ 2R	$MSER_{RV}$ 2E	$MSER_{RV}$ 2RE∆ %	$MSER_{LV}$ 1R	$MSER_{LV}$ 2R	$MSER_{LV}$ 2E	$MSER_{LV}$ 2RE∆ %
MALES	$\bar{x}$	33.2	41.8	37.2	373.8	143.8	209.9	52.5	123.0	158.9	237.4	53.6
	SD	3.9	6.4	7.4	140.9	31.5	43.5	32.5	36.9	27.3	47.1	33.3
FEMALES	$\bar{x}$	35.9	41.5	39.9	334.5	178.2	209.2	66.1	118.9	145.7	222.5	56.2
	SD	7.4	9.7	7.7	147.8	39.7	54.8	37.6	30.1	35.2	51.5	28.9
AGE < 40	$\bar{x}$	33.0	38.3	37.3	375.7	143.5	236.7	69.2	129.6	162.6	253.4	59.0
	SD	5.4	5.6	5.0	150.6	33.3	51.0	35.1	34.1	31.4	46.4	29.4
	r		−0.097	0.304		0.125	0.580			0.430	0.482	
	t		3.591	0.867		8.609	12.046			5.206	11.773	
	P(t)			—		*						
AGE ≥40	$\bar{x}$	37.8	45.5	41.6	308.5	119.8	174.9	53.3	108.3	133.9	193.2	31.0
	SD	7.2	10.4	9.5	132.9	27.5	24.3	36.9	24.7	29.2	31.9	30.7
	r		0.161	0.594		0.125	0.219			0.199	0.340	
	t		3.303	2.129		8.609	8.333			3.733	8.229	
	P(t)						*					
ALL	$\bar{x}$	35.1	41.6	39.2	343.7	132.7	209.4	62.2	120.1	149.6	226.8	55.5
	SD	6.7	8.8	7.6	145.7	32.8	51.4	36.4	31.9	33.4	50.3	30.0
	r		0.209	0.575		0.217	0.569			0.429	0.561	
	t		4.892	2.309		11.206	13.060			6.292	13.300	
	P(t)										**	
M/F	t	1.773	0.134	1.178	0.930	1.650	0.049	1.305	0.395	1.491	1.006	0.265
	P(t)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<40/≥40	t	2.765	3.107	1.966	1.772	2.891	5.754	1.577	2.707	3.507	5.530	0.951
	P(t)			NS	NS		*	NS				NS

Table 24 Three different indices of ventricular distensibility (D 1 D 2 and D-3 see text) in the group of patients subjected to recatheterization at follow up — Symbols 1R 2R and 2E as in Table 18

		D-1						D 2				D 3			
		RV			LV			RV		LV		RV		LV	
		2R	1R	2E	1R	2R	2E	1R	2R	1R	2R	1R	2R	1R	2R
MALES	$\bar{x}$	19 7	11 8	9 3	4 7	6 6	4 3	26 0	11 9	7 4	9 4				
	SD	11 1	6 5	4 6	1 8	2 1	2 6	12 1	5.3	4 9	5 4				
FEMALES	$\bar{x}$	23 5	15 1	9	6 6	9 3	4 3	35 0	13 1	9 7	13 9				
	SD	18 3	9 7	4 2	7 1	10 3	5 6	41 9	7 6	7.3	14 2				
AGE	$\bar{x}$	27 4	16 8	11 3	7 1	10.3	5 6	41 9	15 0	10 8	15 2	1 5	1 1	1 7	1.5
	SD	18 9	9 1	4 4	4 8	5 8	3 3	46 0	8 3	7 5	15 6	0 9	0 6	1.3	1 0
<40	r		0 227	0 205		0 372	0 514	0 193		0 8?0		0 081		0 536	
	t		3 096	3 253		2 971	5 245	3 264		2 119		2 086		0 934	
	$P(t)$													NS	
AGE	$\bar{x}$	16.3	11 0	6 5	4 7	6 3	2 6	20 8	10 3	6.5	9 9	1 5	1 1	1 4	1 8
	SD	10 4	7 8	3 2	2 7	2 7	1 0	9 9	4 0	4.5	7 1	0 6	0 5	0 7	1.5
≥40	r		−0 162	0 537		0 207	0 547	0 018		0 230		0 449		0 354	
	t		1 896	3 396		2 353	8 038	4 861		2 142		3 370		1.351	
	$P(t)$		NS											NS	
ALL	$\bar{x}$	22 4	14 2	9 2	6 0	8 5	4 3	32 5	12 8	9 0	12 7	1 5	1 1	1 6	1 6
	SD	16 5	9 0	4 6	4 1	5 0	2 9	36 2	7 0	6 7	12 5	0 8	0 6	1 1	1.3
	r		0 210	0 409		0 419	0 5?1	0 252		0 734		0 180		0 383	
	t		3 592	4 479		3 770	7 654	4 125		2 929		3 236		0 000	
	$P(t)$													NS	
M/F	t	0 937	1 456	0 043	2 247	2 655	0 039	1 21?	0 655	1.284	1 631				
	$P(t)$	NS	NS	NS			NS	NS	NS	NS	NS				
<40/≥40	t	2 788	2 545	4 555	2 359	3 400	4 707	2 443	2 706	2 503	1 559	0 000	0 000	1 403	0 813
	$P(t)$										NS	NS	NS	NS	NS

atively without any statistically significant differences between the age or sex groups

Comments on systolic and diastolic ventricular function — In addition to the pressure and flow parameters presented in the foregoing some further parameters with reference to ventricular systolic function (*SWI PTI MSER*) and to diastolic function (indices *D-1 D-2* and *D-3* related with ventricular compliance) were derived and evaluated in the present study

The average SWI_{RV} was normalized in all but nine cases in five of which there was increased pulmonary vascular resistance before surgery Considering particularly the fact that in the calculation of the stroke work the contribution of kinetic energy is neglected the right ventricular stroke work may be said to have been remarkably reduced in all cases In about half of the cases equally in both age groups and in the groups of patients with and without increased R_p the SWI_{RV} value reached the preoperative level during exercise The average SWI_{LV} increased significantly as the result of the postoperative increase in systemic flow and arterial blood pressure The total cardiac work per stroke and referred to the body surface area was almost equal before and after surgery and no differences were found between the age groups while the values of males were somewhat higher than those of the females at follow-up It is thus seen that the total cardiac performance in terms of work exerted against pressure did not change substantially but it was more normally distributed between the right and left heart

PTI_R per beat gained normal level as a result of the operation in about half of the patients In all cases PTI_{LV} simultaneously rose to normal level and slightly above normal range in many of the older patients Thus the total cardiac pressure strain in terms of the pressure-time integral increased slightly on the average from preoperative to postoperative condition at rest and further slightly from rest to exercise but the changes were rather small in general Almost equal average total cardiac pressure strain per minute prevailed at rest both preoperatively and at follow-up and during exercise there was presumably no greater stress after than before surgery seeing that the heart rate response during exercise was lowered on the average as a result of surgery in most of the patients in which it was measured (Chapter IX) The left ventricular pressure strain per minute was slightly higher postoperatively than prior to operation PTI_{LV} is known to be one of the best correlates of the myocardial oxygen expenditure at rest although their relations are less strict during exercise, presumably owing to altered fibre kinetics (Gorlin et al 1965) Considering the probably slightly increased oxygen demand of the left ventricle after surgery occurrence of precordial pains and in particular of the anginous syndrome which was not uncommon before surgery was postoperatively rare This may be partly associated with the net effect of the operation on the total tension strain of the heart walls which can be considered to have been smaller after surgery owing to unchanged or reduced total pressure time and markedly reduced heart volume after the operation There was only one male patient aged 41 years who at the time of the follow-up examinations had angina of effort which was even more severe than preoperatively In this case the heart volume and pressure-time indices had been reduced to normal values and the pulse rate response during exercise was likewise normal and lower than preoperatively Therefore it seems that progression of existing coronary disease proper occurred postoperatively in this case

The effective *MSER* is a parameter characterizing one of the aspects of cardiac contractility It has some value in discriminating cases with abnormal ventricular function although its usefulness has been criticized on the grounds of its sensitivity to changes in heart rate and stroke volume (Ross et al 1965) Normally the systolic ejection period is shortened during work and the stroke volume usually increases If abnormal ventricular function is considered to be characterized by lack of these adaptive changes during exercise it would be manifested during work in less than 15 % increase or reduction of *MSER* (Levine et al 1962) which is the quotient of the stroke volume index and the systolic ejection period Such abnormal response was found in five instances with simultaneous abnormal response in *SVI* and *SWI* while in the other cases considerable increase in *MSER* of both ventricles was attendant on exercise on the average $MSER_{RV}$ diminished owing to abolishment of the left-to-right shunt The left ventricular contractile

performance was significantly enhanced as a result of the operation as is apparent from the increased average values of $MSER_{LV}$ and of the mean systolic systemic pressure although there probably was some simultaneous increase of the left ventricular dimensions

The *D-1* index or the distensibility factor of Rowe et al (1961) indicates the ventricular diastolic volume change in relation to the end-diastolic pressure The values for both ventricles are unequal in cases of ASD the factor for the right ventricle being considerably greater than that of the left ventricle as was also evident preoperatively in the present series The direction of the interatrial shunt has been attributed to the difference between the right and left ventricular compliances (e g Dexter 1956) and in conformity with this concept good correlation has been established between the magnitude of the left-to-right shunt and the difference of the distensibility factors (Rowe et al 1961 Petersson 1967) However the difference of the said factors is primarily controlled by the share which the shunt takes from the right ventricular stroke volume which is used in the equation by which the factor is calculated since the other determining factors (SV_{LV} and the difference of $P_{LV_{ed}}$ and $P_{RV_{d}}$) and also the heart rate vary within very narrow limits the correlation mentioned is largely due to purely mathematical causes Nevertheless if the end diastolic pressure is regarded to be an approximation of the change in pressure associated with diastolic ventricular filling the distensibility factor may be considered a measure of the ventricular compliance $\Delta v/\Delta p$ as defined by Guyton (1963) In the present series the distensibility factor of the right ventricle i e the index $D\ 1_{RV}$ was more than three times that of the left ventricle before surgery consistent with the average $Q_p/\dot{Q}$ ratio of 3 4 while it was only about two-thirds of this value at follow-up $D\ 1_{LV}$ indicated increase of the left ventricular compliance at follow up compared to the preoperative value and significantly lower right and left ventricular compliance in the older than in the younger patients both before and after surgery

Another approach in evaluating the ventricular compliance is to apply in the calculations the pressure change indicated by the slope of the slow diastolic ventricular filling curve (Feigenbaum et al 1966) or the stasis wave instead of the end diastolic pressure Experimental studies with heart preparations have shown that the ventricular compliance is comparatively constant at mid-diastole and that the fibre length and intracavital pressure both slowly rise during ventricular diastasis (Buckley et al 1956 Linden and Mitchell 1960) Also combined radiological and haemodynamic observations in intact humans suggest that the changes in compliance during the latter two thirds of the diastolic filling period seem to be rather small (Dodge et al 1966) The index *D-2* calculated as $\Delta t/\Delta p$ from the stroke volume and the slope of the stasis wave consequently constitutes in most cases a rough estimate of the average ventricular compliance during mid and late diastole in spite of the fact that in the presence of an intact atrio-ventricular valve the major portion of ventricular filling most often coincides with the early diastole The $D\text{-}2_{LV}$ index was preoperatively on the average slightly subnormal in the older and normal in the younger patients It increased on the average to normal in the former and to values above normal in the latter This would indicate a downward shift of the average individual ventricular diastolic volume pressure curves (Braunwald and Ross 1963) However this does not seem likely because there is no reason to assume the left ventricular end-diastolic volume to have diminished after surgery while some radiological evidence to the opposite effect exists It follows that the left ventricular compliance has obviously increased after operation The values of the older patients were lower than those of the younger patients as a rule but their changes were more pronounced

The potential plateau pattern of the ventricular diastolic pressure curve which has originally been described in connection with the non compliant heart in constrictive pericarditis (Hansen et al 1951) is indicated and measured by the *D 3* index which represents the ratio of the ventricular end diastolic pressure and the pressure change during the diastolic filling period according to the slope of the stasis wave It is easily calculable from the slope and is also simply obtained as the quotient *D-2/D-1* The plateau pattern is well-known to constitute a characteristic of the low-compliant ventricle *D-3* (or the diastasis index) is normally less than about 2 0 for both

ventricles higher values indicating more or less marked plateau pattern trend usually associated with early diastolic dip In Fig 22 the left ventricular diastasis index at rest on the follow-up studies of the present series has been plotted over the patients age A trend of higher values with increasing age is obvious the relationship being probably curvilinear This pattern suggests lesser left ventricular compliance in older subjects in the present series This phenomenon was associated with increased frequency of other complicating circulatory disturbances The number of cases with $D\text{-}3_{LV}$ higher than 2 0 at follow-up was 11 In all these cases other circulatory abnormalities were also found most common among them (as compared to the respective findings in the entire recatheterized series) atrial fibrillation (3/3) reduced SWI_{LV} during work (4/8) reduced SVI during exercise (3/9) elevated $\overline{P}_{w}$ at rest (1/2) increased AVD at rest (2/6) and reduced S_{O_2} (2/5) No significant changes in the average values of the entire series or of the two age groups were noted after surgery

General comments on the haemodynamic findings at follow up

The net effect of the operation on right atrial function is impossible to judge on the basis of the present data The right atrial a/m ratio remained unchanged (1 34) on the average $\overline{P}_{RA}$ was significantly lowered the volume load was similarly markedly reduced and the dimensions of the right atrium likewise The same applies to evaluation of the left atrial function particularly owing to lack of direct pressure data at follow up The average $\overline{P}_{w}$ at follow up was equal in level to the average $\overline{P}_{LA}$ before surgery and there was no statistically significant difference between these The left atrial a wave was higher in the preoperative records than the a wave in the wedge pressure records at follow-up however this is probably caused by the damping properties of the pulmonary vasculature as has been discussed already Even so the findings suggest the inference that the left atrial function did not appreciably change as a result of the operation as judged merely in terms of pressure The data permit no comparison of the pressure-volume relationships Indirect evidence suggesting improved function of the left atrium after successful correction of the defect is its ability to increase $\overline{P}_{LA}$ (i e., $\overline{P}_{w}$) during exercise according to the requirements of the left ventricular filling without simultaneous marked elevation of $\overline{P}_{RA}$ and systemic venous pressure Suggestions of improvement and normalization of the atrial function are rather furnished by findings other than the haemodynamic ones namely, the electrocardiographic and radiological findings

Taking into account all the haemodynamic parameters of ventricular function presented completely normal findings were encountered in 18 cases (31 %) 14 of them patients younger than 40 years and four older patients (42 % and 16 % of the respective age group)

Abnormality of right ventricular function and of the pulmonary circulation was noted at follow-up in 25 patients (43 %) comprising eight younger and 17 older patients (24 % and 68 % of the respective group) In all these cases increased R_{p} was noted and in less than half of them also signs of abnormal right ventricular function The R_{p} of four younger patients and 17 older patients (12 % and 28 % respectively) changed from a normal preoperative value to an abnormally elevated value after surgery

Abnormality of left ventricular function and of the systemic circulation was found at follow-up in 16 cases (28 %) comprising eight younger

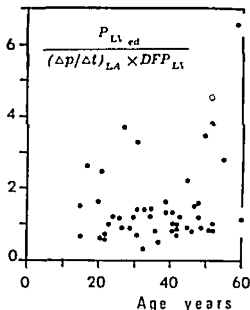


Fig 22 Distensibility index $D\,3_{LV}$ in 49 cases at rest at follow up plotted over age

and eight older patients (24 % and 32 % of the respective group) Arterial hypertension was present in six of these namely in two younger and four older patients (6 % and 16 %, respectively) No signs of abnormal left ventricular function were observed in any of these six cases of mild arterial hypertension The abnormal left ventricular function which was usually only rather slightly deranged was not related to the heart volume before or after surgery It was not related to abnormalities of the pulmonary circulation or right ventricular function either seeing that combined right and left heart abnormality was only established in five instances of which four concered patients of the old age group

It is thus noted that hypertension in the pulmonary or systemic circulation and findings indicating abnormal right ventricular function were more common at follow-up in the group of patients aged 40 years or older than in the young age group while the frequency of abnormalities of left ventricular function was largely equal in both age groups

SYNOPSIS

In all the 129 cases of the present material preoperative principal haemodynamic data were available Reduced S_{O_2} was found in 10 % of the cases only mostly in older patients or in patients with pulmonary vascular disease *AVD* was abnormally high in 33 % most often in older subjects and in cases with atrial fibrillation pulmonary vascular disease or large left to-right shunt The average $\dot{Q}$ was normal but low or abnormally low in 13 % $Q_p/\dot{Q}$ was within the range of 2 0—3 9 in 59 % higher than this in 33.2 % and lower in 7 8 % of the cases lower ratios being more frequent in older patients and in cases with pulmonary vascular involvement $\bar{P}_{RA}$ was slightly elevated in 20 % and elevated up to 10 mm Hg or higher in 6 % mainly in older subjects and in those with atrial fibrillation but not preferentially in association with pulmonary or systemic arterial hypertension $\bar{P}_{LA}$ was higher by 0—4 mm Hg than $\bar{P}_{RA}$ independent of the size of the defect and there was some evidence of a probable causal relationship between impaired left atrial function and hypokinetic systemic circulation P_{PA} was moderately elevated in 31 % and higher than 40 mm Hg (with peak systolic pressure in excess of 50 mm Hg) in 3 % of the case and the prevalence of pulmonary hypertension increased abruptly from less than 10 % at ages under 40 years to more than 30 % in patients older than this R_p was high normal in the older patients but generally low in the patients younger than 40 years Obstructive pulmonary hypertension was found in three patients (two of them 40 years or older) hyperkinetic pulmonary hypertension in two of the older patients and incipient pulmonary vascular disease in three younger and seven older patients

Examinations for residual shunt at follow-up were made by recatheterization with oxymetric and dye dilution studies in 58 cases while these examinations were limited to peripheral dye dilution studies in 71 cases Three instances of residual left-to-right shunt were detected with 30 % 20 % and 10—15 % of pulmonary flow respectively

Recatheterization at follow-up was undertaken in 58 cases 25 of them concerning patients aged 40 years or older Adequate haemodynamic data were obtained in 57 cases and in 53 cases the examination included studies both at rest and during exercise

The heart rate at rest was not significantly changed by the operation Its increase during exercise related to $\dot{V}_{O_2}$ was normal and its slope over $\dot{V}_{O_2}$ was steeper in the older subjects The *AVD* at rest was low in relation to $\dot{V}_{O_2}$ both before and after surgery This hypokinetic pattern was more pronounced in the older patients but an increase resulting in normal value did occur during exercise in most instances Q_p was reduced by the operation in all cases $\dot{Q}$ was normal but low preoperatively and higher but still low at follow-up in relation to $\dot{V}_{O_2}$ especially in older subjects The average slope of the exertional change of $\dot{Q}$ over $\dot{V}_{O_2}$ (the exercise factor) was normal at follow-up in both age groups but it was lower in the old than in the young age group It had an abnormally low value in five older and one younger patient

$\bar{P}_{PA}$ was normalized in three-quarters of the patients after surgery but it increased again during exercise at follow-up particularly in

VIII SUMMARY

Material and outline of the study — ... patients available for foll... of 136 who had been ... ASD of secundum or ... extracorporeal c... surgeon in 19... constitute the ... comprise 98 women ... the sex ratio ... time of oper... (15—60) 101 pati... 40 years (young ... 24 men) and 29 w... old age group ... Ten of the latter were between the ages of 50 and 60 years ... preoperative ... the same examination ... after surgery in 58 cases including ... of ... group and of ... exercise ... 53 cases ... phonocardiographic records ... of 15 ... preoperatively and of all 129 after surgery. Determinations of submaximal physical working capacity were made in 38 cases before and in all 129 after the operation. Dye dilution curves were available of nearly all cases preoperatively and of all 129 postoperatively. Control data consisting of radiological electrocardiographic exercise test and catheter examination results obtained with altogether about 200 healthy subjects of different sex and ages served for reference in the study. The follow up times of the series averaged 29.6 months.

Surgery — Two deaths occurred in the original surgical series of 136 consecutive operations with the aid of cardio pulmonary bypass (2.2 %) but the last 109 of them were entirely without fatalities. In 31 % of the series postoperative non surgical complications were noted about twice as frequently in the old as in the young age group. Commonest were arrhythmias (63 % of all non surgical complications) their frequency in the old age group was nearly three times that in the young age group. Late mortality is made up of two cases with causes of death unrelated to surgery.

Medical history and physical findings. — On analysis of the ages at which various symptoms appeared in the entire series various dysrhythmias were found to increase with age their onset most numerously concentrated in the third decade of age. this development was completed by the appearance of atrial fibrillation at ages over 40. The same behaviour was displayed by symptoms of heart failure. Their appearance was experienced by the patients as a distinct worsening of condition. In those of the subjects who entered class III of N.Y.H.A. (about 20 % of the series) such deterioration took place between the ages of 30 and 50. The frequency of most kinds of the various symptoms was less than 15 % at follow up. Improvement of functional capacity by one class or more was noted in 84 % of the patients in the old age group and in 94 % of the younger ones when there had been reduced capacity before surgery despite the patient's tendency to overrate their capacity before the operation and to underrate it after the operation.

The auscultatory findings were abnormal before surgery in every case they included fixed and/or widely split second sound in about 90 % systolic murmur in 100 % and tricuspid inflow murmur in one third of the cases. Postoperatively pulmonary systolic murmur was heard without relation to systolic pulmonary valvular gradient or to heart shape in one third of the cases and 12 % presented a second sound which appeared fixed to the ear. Accentuated and palpable pulmonary second sound possessed some value in clinical discrimination of cases with and without increased pulmonary vascular resistance. Of the

cases with residual left-to-right shunt three in all one with large shunt displayed the same symptoms and signs as before surgery the other two who had a minor shunt had improved except for tricuspid inflow murmur in one of them

Phonocardiography — Auscultatory split of the first sound which was common both before and after the operation particularly in the old age group was best related to delayed and often accentuated ejection component of the first sound in the phonocardiogram it tended to become less common with lapse of time after surgery The split of the second sound exceeded 30 msec preoperatively and increased by less than 20 msec during inspirium Postoperatively it was less than 30 msec and/or increased by more than 20 msec at inspiration The clinical significance of the second sound in distinguishing between cases with and without residual shunt was impeded by false normal findings in two of three cases with residual shunt and by false abnormal finding in one case with complete right bundle branch block

Electrocardiography — Sinus rhythm was preoperatively present in 99 % of the patients in the young and in 61 % of those in the old age group atrial fibrillation occurring in most of the rest The respective figures at follow up were 100 % and 86 % Slight atrial conduction defects were rather common before and particularly after surgery they were probably due both to the anomaly itself and to surgical trauma Prolonged P-R time was noted in 8 % of the cases preoperatively and in 6 % at follow-up The QRS duration was 120 msec or more in 17 % of the young age group and in 32 % of the old age group before and in 4 % and 17 % respectively after the operation Signs of right atrial overload were present in 6 % of the cases preoperatively high P in lead II often occurring in association with pulmonary hypertension while they were absent after surgery Signs of left atrial overload were pre operatively found in 11 % and at follow up in 15 % of the cases The initial P forces in lead V_1 diminished after operation while the terminal forces changed very little on the average The atrial mean frontal vectors and their right and left atrial components moved slightly left wards after operation Left axis deviation and counterclockwise frontal QRS sÊ loop occurred in three cases (2 3 %) the commonest finding being right axis deviation with clockwise figure-eight loop The commonest preoperative morphologies of QRS in lead V in the young age group were rsR, rsr' and rs in the old age group rsR rsr and rR QRS was normal in 5 % preoperatively and in 20 % after surgery with normalcy commoner in the young age group The most constant change associated with successful correction of the defect was narrowing of the s deflection in lead V or V_6 this was observed in 94 % of the cases one case with residual shunt included among them however Poor correlation was noted to exist between haemodynamic and electrocardiographic findings as a rule particularly as regards atrial function The right ventricular pressure time integral displayed reasonable positive correlation on presence of right ventricular hypertrophy pattern in lead V_1

Radiology — Normal heart volume was preoperatively recorded in 19 % of the cases and the heart volume was in excess of 800 ml/m² *BSA* in 14 % of the cases The corresponding figures at follow up were 51 % and 1 % the heart volume had been reduced from the preoperative value by 20 % on the average in the young and by 27 % in the old age group In all cases hilar dance was seen preoperatively which occurred at follow-up in six cases including the three with residual shunt Preoperative subtle signs of pulmonary interstitial oedema were found in 31 % of the cases before and in 15 % after the operation The right heart and pulmonary artery were enlarged before surgery in the majority of the cases and the left atrium in 29 % The left ventricle appeared small in 27 % while the relative cros sectional area of the aortic arch (in cm²/m² *BSA*) considered in relation to age was significantly smaller than normal on the average The operation had produced slight but distinct changes in the left ventricle and aorta which were reciprocal to the marked reduction in size of the right heart and pul monary artery All these alterations parallelled the changes in blood flow in the respective parts of the central circulation

Exercise test — The submaximal work capacity at 150/min pulse rate (PWC_{150}) in

sitting position was preoperatively abnormally low related to body weight in 13 % of the cases and related to heart volume in 30 % if the effect of the shunt on the heart volume is taken into account At follow-up 70 % of the cases presented significantly increased PWC_{170}. The average PWC_{170} at follow-up was normal in both age groups low values being commoner among the older patients It was abnormally low in relation to body weight in 7 % and, in relation to heart volume in 41 % of the cases suggesting irreversible myocardial changes of rather common occurrence Reduced work capacity was most often associated with complications such as pulmonary vascular disease atrial fibrillation or residual shunt The findings in the exercise test and the functional classification according to NYHA showed fair average agreement in spite of wide scatter it was obvious that the patients tended to overrate their capacity before operation and to underrate that after the operation

Lung function tests. — The vital capacity *(VC)* was normal preoperatively in 54 % and after surgery in 74 % of the cases more commonly in the young age group Significant improvement of VC at follow-up was presented by 25 % of the cases equally in both age groups improvement was commonest in cases with marked postoperative reduction of heart volume less common in those with pulmonary hypertension The forced expiratory volume *(FEV₁)* was normal before surgery in 58 % at much higher frequency in the young than in the old age group and at follow-up in 70 % of the cases It was not related to haemodynamic or radiological findings

Haemodynamic findings — The arterial oxygen saturation was preoperatively reduced in 10 % and at follow-up in 5 %, particularly in the middle-aged patients with pulmonary vascular disease The systemic flow *($\dot{Q}$)* was abnormally low in 13 % and the arteriovenous oxygen difference *(AVD)* was abnormally high in 33 % of the cases preoperatively most often in the old age group and in cases with atrial fibrillation pulmonary vascular disease or large left-to-right shunt Recatheterization at follow-up still revealed low *AVD* in relation to oxygen uptake *(V_{O_2})* particularly in the old age group but the values normalized on the average during moderate supine exercise Likewise $\dot{Q}$ was still low at follow-up in relation to V_{O_2} especially in the old age group The slope of the change in $\dot{Q}$ over V_{O_2} (the "exercise factor") was normal in all but five older patients and one younger patient The pulmonary-to-systemic flow ratio *($\dot{Q}_p/\dot{Q}_s$)* was less than 2.0 in 7.8 % and in excess of 3.9 in 33.2 % of the cases low ratios being more common in the old age group and in cases with pulmonary vascular disease A residual shunt was detected at follow up by recatheterization or by dye dilution technique in three cases (2.3 %) in two of which the shunt was insignificant in magnitude

The right atrial pressure *($\overline{P}_{RA}$)* was elevated to 10 mm Hg or higher in 6 % of the cases before surgery mainly in the old age group and in cases with atrial fibrillation but not preferentially in association with pulmonary or systemic hypertension High preoperative $\overline{P}_{RA}$ was not associated with any signs of left ventricular dysfunction or failure at follow up It was inferred that impaired effectiveness of atrial contraction may be responsible for elevated right atrial mean pressure up to normal level of the left ventricular filling pressure which is often over 10 mm Hg in absence of any abnormality in the left ventricular function There was some evidence of impairment of left atrial function with increasing age and of probable causal relationship between depressed atrial function and hypokinetic systemic circulation The left atrial mean pressure was higher by 0—4 mm Hg than the right atrial mean pressure independent of the size of the defect The average $\overline{P}_{RA}$ was found to be reduced after surgery while the average wedge mean pressure *($\overline{P}_W$)* remained on the preoperative level of the average left atrial mean pressure *($\overline{P}_{LA}$)* $\overline{P}_{RA}$ and $\overline{P}_W$ both increased during work at follow-up more strongly in the old age group which at the same time displayed smaller increase in stroke volume index *(SVI)* but the elevation was normal as related to $\dot{Q}$ and to the patient's age in all but five instances which represented both age groups equally Only one of the five displayed simultaneously with abnormally increased filling pressure a drop in *SVI* suggesting left ventricular failure

The preoperatively abnormally large right ventricular *SVI* and abnormally small left ventricular *SVI* changed reciprocally and both were normal on the average at follow-up with lower values in the old age group *SVI* as a rule increased during work at follow-up (by 25 % on the average), but it remained unchanged or was reduced in seven instances representing both age groups equally The stroke work index *(SWI)* changed in a fashion similar to that of *SVI* Observations concerning the pressure-time index *(PTI)* suggested reduction of the total cardiac tension strain after the operation The left ventricular mean systolic ejection rate *(MSER)* indicated on the average improvement of left ventricular systolic function in all but five cases which represented both age groups equally Minor abnormalities in parameters of right ventricular function at follow-up were more common in the old age group but in respect of those of the left ventricle both age groups were alike The parameters describing the ventricular compliance revealed reduction in right ventricular compliance and increase in left ventricular compliance after surgery and a tendency of the latter to become less with increasing age The inference was drawn from this that one additional factor contributing to greater disability with increasing age in ASD would seem to be the result of gradually diminishing left ventricular compliance and of simultaneous progressively poorer filling assistance furnished by the left atrium

The prevalence of pulmonary hypertension was less than 10 % at ages under 40 years and higher than 30 % at ages over 40 The pulmonary artery pressure was normalized after operation in three-quarters of the cases The preoperatively elevated pulmonary vascular resistance index (R_{p_i}) showed equally common reduction and increase after surgery independent of the severity of the pulmonary vascular disease In numerous cases in the old age group R_p increased from preoperatively normal values to abnormally high values at follow-up which might be considered a sign of pulmonary vascular disease still in progress after surgery

REFERENCES

Adams C W A reappraisal of life expectancy with atrial shunts of the secundum type Dis Chest. 48 356 1965

Ainger L. E. and Pate J W Ostium secundum atrial septal defects and congestive heart failure in infancy Amer J Cardiol. 15 380 1965

Anselmi G., Muñoz Armas S., Salazar A., Anselmi A., DePisani F., and Blanco P Electrocardiographic patterns of right atrial overloading in some congenital heart conditions. Amer J Cardiol. 21 628 1968

American College of Chest Physicians Report of the Committee on Cardiovascular Surgery Survey of surgical treatment of atrial septal defects Dis Chest. 43 447 1963

Amorim, D deS Marshall, H W., Donald D E., and Wood E. H Effect of atrial septal defects on hemodynamic alterations caused by acute partial obstruction of pulmonary artery Circulat Res 10 227 1962.

Ariza Mendoza F., and Woolf C R. The value of pulmonary function studies in the assessment of patients for cardiac surgery Canad med. Ass. J 91 1250 1964

Arnfred E. A clinical and hemodynamic evaluation of the results of surgical correction of atrial septal defect. J cardiovasc Surg (Torino) 8 93 1967a

Arnfred E. The significance of the radiological examination in evaluating the results of surgical treatment of atrial septal defects. J cardiovasc. Surg. (Torino) 8 230 1967b

Arvidsson H. Angiocardiographic measurements in congenital heart disease in infancy and childhood I The size of the ascending aorta. Acta radiol Diagn. 1 981 1963

Assman, H Die klinische Rontgendiagnostik der inneren Erkrankungen. Ed 4 Vogel, Leipzig 1928

Axen, O., Lindgren, E., and Malmström, G Till kannedom om mätfelen vid Liljestrand Lysholm Nylin Zachrissons metod for hjartvolymbestämning. Nord Med 29 592, 1946

Aygen, M M., and Braunwald, E. The splitting of the second heart sound in normal subjects and in patients with congenital heart disease Circulation 25 328 1962.

Bailey C P., Downing D F., Geckeler G D., Likoff W., Goldberg, H., Scott, J C., Janton, O., and Redondo-Ramirerez, H P.. Congenital interatrial communication clinical and surgical considerations with a description of a new surgical technic atrio-septo-pexy Ann. intern. Med. 37 888 1952.

Barber J M., Magidson, O., and Wood P Atrial septal defect. Brit. Heart J 12 277 1950

Barclay R. S Anomalous pulmonary veins with or without anomalous drainage p 79 In Modern trend. in cardiac surgery Ed. by H.R.S. Harley Butterworths, London 1960

Barger J D., Edwards, J E., Parker R. L., and Dry T J Atrial septal defect presentation of a case with obstructive pulmonary vascular lesions caused by metastatic carcinoma. Proc. Mayo Clinic 23 182, 1948

Barker J M., and Valencia, F.. The precordial electrocardiogram in incomplete right bundle branch block. Amer Heart J 38 376 1949

Beck, W., Swan, H. J C., and Burchell, H. B., and Kirklin, J W.. Pulmonary vascular resistance after repair of atrial septal defects in patients with pulmonary hypertension. Circulation 22 938 1960

Bedford, D E.. The anatomical types of atrial septal defect Their incidence and clinical diagnosis. Amer J Cardiol. 6 568 1960

Bedford, D E. Atrial septal defect. Proc roy Soc. Med 54 779 1961

Bedford D E., Papp C., and Parkinson, J Atrial septal defect. Brit. Heart J 3 37 1941.

Bedford, D E., and Sellors, T H.. Atrial septal defect. p. 138 Modern trends in cardiology Ed. by A. Morgan Jones, Butterworths, London, 1960

Beller B M. Influence of abnormalities of ventricular excitation on right to left shunt flows in atrial septal defect. Amer J Cardiol. 20 583, 1967

Bender F.. Diagnose und Differentialdiagnose von Links-Rechts-Shunts mit Farbstoffverdunnungskurven bei peripherer Injektionstechnik. Z. Kreisl.-Forsch. 53 264, 1964.

Bender F., and Koch, F.. Reaktive Hyperamie als Mittel zur Verbesserung der Indikatorverdunnugmethode in der Diagnostik von Rechts Links-Shunts. Z. Kreisl. Forsch. 49 129 1960

Berkson, J and Boothby W.M Studies of the energy of metabolism of normal individuals Amer J Physiol. 121 669 1938

Berson, A S and Pipberger H V The low frequency response of electrocardiograph a frequent source of recording errors Amer Heart J 71 779 1966

Berson A S and Pipberger H V Electrocardiographic distortions caused by inadequate high-frequency response of direct writing electrocardiographs Amer Heart J 74 208 1967

Besterman, E Atrial septal defect with pulmonary hypertension. Brit. Heart J 23 587 1961

Bevegård S Holmgren, A and Jonsson, B The effect of body position on the circulation at rest and during exercise with special reference to the influence on the stroke volume Acta physiol. scand 49 279 1960

Bjork V O Holmdahl M H son, Jonsson, B and Petersson, P O Hypothermia with coronary perfusion for the correction of atrial septal defects with or without abnormal pulmonary veins Acta chir scand 119 317 1960

Bjork, V O Crafoord, C., Jonsson, B Kjellberg S R. and Rudhe U Atrial septal defects A new surgical approach and diagnostical aspects Acta chir scand 107 499 1954

Bjork, V O Holmdahl M. H son, Jonsson, B and Petersson P O Hypothermia with coronary perfusion for the correction of atrial septal defects with or without abnormal pulmonary veins Acta chir scand 119 317 1960

Blount, S G Jr Bachum O J and Gensini G The persistent ostium primum atrial septal defect Circulation 13 499 1956

Blount, S G Jr Gensini G and McCord M C The pulmonary hemodynamic pattern in patients with atrial septal defects before and after closure J Lab clin Med 42 785 1953

Blount, S G Jr Munyan, E A. Jr., and Hoffman, M. S Hypertrophy of the right ventricular out flow tract Amer J Med 22 784 1957

Blount, S G Jr Swan, H Gensini G and Mc Cord, M C Atrial septal defect Clinical and physiological response to complete closure in five patients Circulation 9 801 1954

Bohlau, V Bohlau, E Herbst, M. and Haack, G Die Untersuchung des Erholungsquotienten in der Herzdiagnostik, insbesondere zur Objektivierung der Erfolgsbeurteilung nach Herzoperationen Med Welt (Berl.) 46 2442, 1960

Bousvaras G A., Palmer W H Sekelj P and Mc Gregor M Comparison of central and peripheral injection sites in the estimation of cardiac output by dye dilution curves Circulat. Res 12 317 1962

Bowles, L T Hallman, G L and Cooley D A. Open heart surgery on the elderly Results in 51 patients sixty years of age or older Circulation 33 540 1966

Brannon E S Weens H. S and Warren, J V Atrial septal defect. Study of hemodynamics by the technique of right heart catheterization. Amer J med Sci 210 480 1945

Braunwald E Brockenbrough, E. C Frahm, C J., and Ross, J Jr Left atrial and left ventricular pressures in subjects without cardiovascular disease Circulation 24 267 1961

Braunwald E Donoso E. Sapin, S O and Grishman, A. Right bundle branch block. Hemodynamic vectorcardiographic and electrocardiographic observations Circulation 13 866 1956

Braundwald, E. and Frahm, C J Studies on Starling's law of the heart IV Observations on the hemodynamic functions of the left atrium in man. Circulation 24 633 1961

Braundwald, E and Ross J The ventricular end diastolic pressure Appraisal of its value in the recognition of ventricular failure in man. Amer J Med 34 147 1963

Braundwald E. Sonnenblick, E H Ross J Glick, G and Epstein, S E An analysis of the cardiac response to exercise Circulat Res 20 Suppl. 1 44 1967

Braunwald N S and Braunwald, E The effects of surgical abolition of left to right shunts on the pulmonary vascular dynamics of patients with pulmonary hypertension. Circulation 26 1270 1962.

Broadbent, J C and Wood E H Indicator dilution curves in acyanotic congenital heart disease Circulation 9 890 1954

Brock, R and Ross, D N The sinus venosus type of atrial septal defect. Guy's Hosp Rep 108 291, 1959

Brody D A., Arzbaecher, R C Woolsley M D., and Sato T The normal atrial electrocardiogram morphologic and quantitative variability in bipolar extremity leads Amer Heart J 74 4 1967

Brotmacher L., and Deuchar D C The sytemic blood flow in congenital heart disease with an examination of the validity of the cardiac index. Clin Sci. 15 441 1956

Bruce R A and Rodgers, D L Quantitative effects of medical and surgical treatment of mitral stenosis on exercise tolerance Amer J Med 15 35 1953

Bruce R A Rowell, L B Blackmon J R., and Doan, A Cardiovascular function tests Heart Bull 14 9 1965

Buckley N M. Sidky M and Ogden, E. Factors altering the filling of the isolated left ventricle of the dog heart. Circulat. Res. 4 148 1956

Burch G E. and DePasquale N P Electrocardiography in the diagnosis of congenital heart disease Lea & Febiger Philadelphia 1967

Burchell, H B Studies in pulmonary hypertension in congenital heart disease Brit. Heart J 21 255 1959

Burchell, H B DuShane J W., and Brandenburg R. O The electrocardiogram of patients with atrioventricular cushion defects (defects of the atrioventricular canal) Amer J Cardiol 6 575 1960

Burrett, J B and White P D Large interauricular septal defect with particular reference to diagnosis and longevity Amer J med Sci 209 355 1945

Cabrera, C E., and Monroy J R Systolic and diastolic loading of heart electocardiographic data Amer Heart J 43 669 1952.

Campbell, M., Neill C and Suzman, S The prognosis of atrial septal defect Brit. med J 1 1375 1957

Campbell, M and Polani P E. Factors in the aetiology of atrial septal defect. Brit. Heart J 23 477 1961

Canada W J Goodale F., Jr., and Currens J H Defect of the inter atrial septum with thrombosis of the pulmonary artery New Engl. J Med 248 309 1953

Carlgren, L E The incidence of congenital heart disease in children born in Gothenburg 1941—1950 Brit Heart J 21 40 1959

Carlgren, L. E. Results of closed heart operation for atrial septal defect. Acta chir scand 122 371 1961

Caro C G Harrison, G K., and Mognoni P Pressure wave transmission in the human pulmonary circulation Cardiovasc Res 1 91 1967

Carter S A. Bajec D F., Yannicelli E and Wood E H Estimation of left to right shunt from arterial dilution curves J Lab clin. Med 55 77 1960

Castellanos, A., and Hernandez, F A. Size of ascending aorta in congenital cardiac lesions and other heart diseases. Acta radiol. Diagn. 6 49 1967

Castle R F Variables affecting the splitting of the second heart sound in atrial septal defect. Amer Heart J 73 468 1967

Chapman C B Fisher J N., and Sproule B J Behaviour of stroke volume at rest and during exercise in human beings J clin. Invest 39 1208 1960

Chiong, M A Interatrial septal defect and longevity Canad med. Ass. J 83 1012 1960

Christensen, E H Minutenvolum und Schlagvolum des Herzens wahrend schwerer korperlicher Arbeit. V Mitteilung Arbeitsphysiologie 4 470 1931

Cohen M G and Pastor B H Delayed cardiac arrhythmias following non cardiac thoracic surgery Dis Chest 32 435 1957

Cohn L. H Morrow A. G., and Braunwald E. Operative treatment of atrial septal defect clinical and haemodynamic assessments in 175 patients. Brit Heart J 29 725 1967

Colmers R A Atrial septal defect in elderly patients. Amer J Cardiol 1 768 1958

Committee of Medical Ratings of Physical Impairment. Guides to the evaluation of permanent impairment. The cardiovascular system J Amer med Ass. 172 1049 1960

Connolly D C., Tompkins R. G Lev R., Kirklin, J W., and Wood E. H Pulmonary artery wedge pressures in mitral valve disease relationship to left atrial pressures. Proc Mayo Clin. 28 78 1953

Cooley D A Hallman, G L., and Hammam A S Congenital cardiovascular anomalies in adults. Results of surgical treatment in 167 patients over age 35 Amer J Cardiol. 17 303 1966

Costa A Studio sulla morfogenesi e la fisiopatologia dei difetti congeniti del setto interatriale del cuore Cuore e Circol 15 263 1931 quoted by Davidsen 1960

Coulshed N., and Littler T R. Atrial septal defect in the aged Brit. med J 1 76 1957

Cournand A., Motley H L. Himmelstein, A., Dresdale D., and Baldwin, J Recording of blood pressure from the left auricle and the pulmonary veins in human subjects with interauricular septal defects Amer J Physiol. 150 267 1947

Craig, R. J., and Selzer A Natural history and prognosis of atrial septal defect Circulation 37 805 1968

Daicoff G R., Brandenburg R. O and Kirklin, J W Results of operation for atrial septal defect in patients forty five years of age and older Circulation 35 Suppl 1 143 1967

Dalen, J E Haynes F W and Dexter L Life expectancy with atrial septal defect. J Amer med Ass 200 442 1967

Davidsen, H G Atrial septal defect an investigation into the natural history of a congenital heart disease (Thesis Copenhagen) Munksgaard Copenhagen 1960

Davies H and Gazetopoulos N Haemodynamic changes on exercise in patients with left to right shunts Brit. Heart J 28 579 1966

Davies, D H Pryor R and Blount, S G., Jr Electrocardiographic changes in atrial septal defect *following surgical correction. Brit. Heart J* 22 274, 1960

Denzler Rigotti B Recherches sur le volume sanguin central Cardiologia (Basel) 39 12 1961

DeOliveira J M., and Zimmerman, H A The electrocardiogram in interatrial septal defects and its correlation with hemodynamics Amer Heart J 55 369 1958

DePasquale N P., and Burch, G E Analysis of the RSR complex in lead V Circulation 28 362, 1963

Derra E. Grosse Brockhoff F., and Loogen F Der Vorhofseptumdefekt. Ergebn. inn Med.-Kinderheilk 22 211 1965

Dexter L Atrial septal defect Brit Heart J 18 209 1956

Dexter L Dow J W Haynes F W Whittenberger J L Ferris B G Goodale W T and Hellems H K Studies of the pulmonary circulation in man at rest Normal variations and the interrelations between increased pulmonary blood flow elevated pulmonary arterial pressure and high pulmonary capillary pressures J clin Invest 29 602 1950

Dexter L Whittenberger J L Haynes F W Goodale W T Gorlin R and Sawyer C G Effect of exercise on circulatory dynamics of normal individuals J appl Physiol 3 439 1951

Dodge H T Sandler H Baxley W A and Hawley R R Usefulness and limitations of radiographic methods for determining left ventricular volume Amer J Cardiol 18 11 1966

Dollery C T West, J B Wilken D E L Goodwin J E F and Hugh Jones H Regional pulmonary blood flow in patients with circulatory shunts Brit Heart J 23 225 1961

Donald K W Bishop J M Cumming G and Wade O L The effect of exercise on the cardiac output and circulatory dynamics of normal subjects Clin Sci 14 37 1955

Donald K W Bishop J M and Wade O L A study of minute to minute changes of arterio venous oxygen content difference oxygen uptake and cardiac output and rate of achievement of a steady state during exercise in rheumatic heart disease J clin Invest 33 1146 1954

Dotter C T and Steinberg I Angiocardiographic measurement of normal great vessels Radiology 52 353 1949

Dow J D The radiological diagnosis of the sinus venosus type of atrial septal defect Guy s Hosp Rep 108 305 1959

Drabkin D L Schmidt C F Bruner, H D and Pennes H H The direct spectrophorometric determination of the saturation of hemoglobin in the arterial blood of man Amer J med Sci 208 135 1949

Dreifus L S Bender S Goldberg H and Downing D F The electrocardiogram in atrial septal defect Dis Chest 36 521 1959

DuBois D and DuBois E F A formula to estimate the approximate surface area if height and weight be known Arch intern Med 17 863 1916

DuShane J W Weidman W H Brandenburg, R O and Kirklin J W Differentiation of interatrial communications by clinical methods Ostium secundum ostium primum common atrium and total anomalous pulmonary venous connection Circulation 21 363 1960

Efskind L Husom O Mollestad O and Storstein O Atrieseptumdefekter behandlet under aapen kardiotomi T norske Laegeforen 79 1013 1959

Eisenberg R and Hultgren N R Phonocardiographic features of atrial septal defect Circulation 20 490 1959

Ekelund L G and Holmgren, A Central hemodynamics during exercise Circ Research 20 Suppl 1 33 1967

Ellis F H Jr Brandenburg R O and Swan H J C Defect of the atrial septum in the elderly Report of successful surgical correction in five patients sixty years of age or older New Engl J Med 262 219 1960

Ellis F R Greaves M and Hecht H H Congenital heart disease in old age Interauricular septal defect with mitral and tricuspid valvulitis. Amer Heart J 40 154 1950

Feigenbaum H Campbell R W Wunsch Ch M and Steinmetz E F Evaluation of the left ventricle in patients with mitral stenosis Circulation 34 462 1966

Feruglio G A An intracardiac sound generator for the study of the transmission of heart murmurs in man Amer Heart J 63 232 1962

Fisher J M Wilson W R and Theilen E O Recognition of congenital heart disease in the fifth to eighth decades of life Diagnostic criteria and natural history Circulation 25 821 1962

Fleming H A Differential lung function in atrial septal defect. Circulation 19 856 1959

Fouché R F Beck, W and Schrire V The roentgenologic assessment of the degree of left to right shunt in secundum type atrial septal defect Amer J Roentgenol 89 254 1963

Freeman A R and Levine S A The clinical significance of the systolic murmur Ann Intern Med 6 1371 1933

Frick M H Observations on hemodynamic factors and left heart performance in essential hypotension Acta med scand 172 Suppl 380 1962 (Thesis Helsinki)

Frick M H Punsar S and Somer T The spectrum of cardiac capacity in patients with nonbstructive congenital heart disease Amer J Cardiol 17 20 1966

Friedman C E Heart volume myocardial volume and total capacity of the heart cavities in certain chronic heart diseases Acta med scand 140 257 1951

Fry D L Noble F W and Mallos A J An evaluation of modern pressure recording systems Circulat Res 5 40 1957

Gault J H Morrow A G Gay W A and Ross J, Jr Atrial septal defect in patients over age of forty years Clinical and hemodynamic studies and effects of operation Circulation 37 261 1968

Gay B B Jr and Franch R H Pulsation in the pulmonary arteries as observed with roentgenoscopic image amplification observations in patients having increased pulmonary blood flow Amer J Roentgenol 85 1025 1961

Gebhardt, W Eine neue Methode der rontgenologischen Herzvolumenbestimmung mit Hilfe des simultanen Schichtverfahrens im Vergleich zu den bisher ublichen Methoden Klin Wschr 35 1119 1957

Gibbon, J H., Jr The application of a mechanical heart and lung apparatus in cardiac surgery Minn Med 37 171, 1954.

Gibson, R The cause of the continued left to-right shunt in A S D when the right atrial pressure is high. Brit. Heart J 20 267 1958

Gooch A S Calatayud J B Gorman, P A. Saunders J L and Carceres, C A. Lefward shift of the terminal P forces in the ECG associated with left atrial enlargement. Amer Heart J 71 727 1966

Gordon, R., Neilson G., and Silverstone H Electrocardiographic P wave and atrial weights and volumes Brit. Heart J 27 749 1965

Gorlin R. Cohen, L S Elliott W C Klein, M D and Lane F J Effect of supine exercise on left ventricular volume and oxygen consumption in man. Circulation 32 361 1965

Graettinger J S., Carleton, R A., and Muenster J J Circulatory consequences of changes in cardiac rhythm produced in patients by transthoracic direct current shock J clin. Invest. 42 939 1963

Granath, A Mitral valvulotomy A clinical and hemodynamic pre- and postoperative study Acta med scand 178 Suppl 433 1965 (Thesis Stockholm)

Granath, A., Jonsson B., and Strandell, T Circulation in healthy old men Studied by right heart catheterization at rest and during exercise in supine and sitting position. Acta med. scand. 176 425 1964

Granath A and Strandell, T Relationships between cardiac output stroke volume and intracardiac pressures at rest and during exercise in supine position and some anthropometric data in healthy old men Acta med scand 176 447 1964.

Grant, C., Bunnell I L and Greene D G The reservoir function of the left atrium during ventricular systole An angiographic study of atrial stroke volume and work. Amer J Med 37 36 1964

Grant, R. D and Estes E H Jr Spatial vector electrocardiography Blakiston New York 1951

Grimby G Exercise in man during pyrogen induced fever Scand J clin. Lab Invest. 14 Suppl. 67 1962 (Thesis Goteborg)

Gross, R. E. Watkins E., Jr., Pomeranz, A A., and Goldsmith E. I A method for surgical closure of interauricular septal defects. Surg Gynec Obstet. 96 1 1953

Grosse Brockhoff F Loogen, F and Wolter H H Analyse von Vorhofdruckkurven zur Grossenbeurteilung von Vorhofseptumdefekten. Z Kreisl-Forsch 46 854 1957

Gustafson, J E., and Friedenberg, M. J Evaluation of left heart disease by statistical analysis of aortic parameters and transverse cardiac diameter Amer Heart J 69 479 1965

Guyton, A C Circulatory physiology Cardiac output and its regulation. Saunders Philadelphia and London, 1963.

Hakkila J., Siltanen, P., and Pietilä, K. Incidence of heart disease in a mass radiophotography survey of adults Ann. Med intern. Fenn. 54 149 1965

Halonen, P I Die Koronararterien des menschlichen Herzens Acta soc med fenn. Duod A XXI, 1938 (Thesis, Helsinki)

Hansen A T Pressure measurement in the human organism. Acta physiol. Scand. Suppl. 69 1949 (Thesis, Copenhagen)

Hansen A. T., Eskildsen, P., and Goetzsche H Pressure curves from the right auricle and the right ventricle in chronic constrictive pericarditis Circulation 3 881 1951

Hanson J S Tabakin, B S and Levy A M Comparative exercise-cardiorespiratory performance of normal men in the third fourth and fifth decades of life Circulation 37 345 1968

Haroutunian, L. M., Neill, C A., and Otis A B The contour of the right atrial wave in 27 cases of atrial septal defect and in other cardiac conditions. Bull. Johns Hopk. Hosp 102. 146 1958

Harris, J A., and Benedict, F G A biometric study of basal metabolism in man. Carnegie Inst., Wash Publ nr 279 1919

Harvey R. M., Smith, W M., Parker J O., and Ferrer M I. The response of the abnormal heart to exercise Circulation 26 341, 1962.

Hauwaert, L. van der The effect of the Valsalva maneuver on the splitting of the second sound. Acta cardiol (Brux.) 19 518 1964

Hawthorne E W., Brownlee G V., and Spellman, M W Prophylaxis against acute pulmonary edema and death with aortic insufficiency following renal artery constriction afforded by prior construction of an atrial septal defect. Amer J Physiol. 185 479 1956

Haywood L J., and Selvester R H. Analysis of right and left atrial vectorcardiograms. Timed records of 100 normal persons Circulation 33 577 1966

Hazan, S J Psychiatric complications following cardiac surgery I A review article J thorac cardiovasc Surg. 51 307 1966

Heikkila J Mitral incompetence as a complication of acute myocardial infarction. Acta med scand 182 Suppl. 475 1967 (Thesis, Helsinki)

Heim de Balsac M. R (In discussion) Arch. Mal Coeur 32 199 1939

Hertle F H., Abel, H., and Heidl, H. Die Abhängigkeit der Arbeitsfrequenz und Frequenzsteigerung bei *einer submaximalen* Ergometerbelastung von Alter und Gewicht Int. Z. angew Physiol. 24 320 1967

Dexter L Atrial septal defect. Brit. Heart J 18 209 1956

Dexter L., Dow J W Haynes F-W., Whittenberger J L Ferris B G Goodale W T and Hellems H K. Studies of the pulmonary circulation in man at rest. Normal variations and the interrelations between increased pulmonary blood flow elevated pulmonary arterial pressure and high pulmonary capillary pressures. J clin. Invest. 29 602, 1950

Dexter L. Whittenberger J L. Haynes F W., Goodale W T., Gorlin R and Sawyer C G Effect of exercise on circulatory dynamics of normal individuals. J appl. Physiol. 3 439 1951

Dodge H T Sandler H. Baxley W A. and Hawley R R. Usefulness and limitations of radiographic methods for determining left ventricular volume Amer J Cardiol. 18 11 1966

Dollery C T West, J B Wilken, D E L., Goodwin, J E F and Hugh-Jones, H Regional pulmonary blood flow in patients with circulatory shunts Brit Heart J 23 225 1961

Donald, K. W Bishop J M Cumming G and Wade O L The effect of exercise on the cardiac output and circulatory dynamics of normal subjects Clin. Sci. 14 37 1955

Donald K. W., Bishop J M and Wade O L. A study of minute to minute changes of arteriovenous oxygen content difference oxygen uptake and cardiac output and rate of achievement of a steady state during exercise in rheumatic heart disease J clin. Invest. 33 1146 1954

Dotter C T and Steinberg, I Angiocardiographic measurement of normal great vessels. Radiology 52 353 1949

Dow J D The radiological diagnosis of the sinus venosus type of atrial septal defect. Guy's Hosp Rep 108 305 1959

Drabkin D L Schmidt, C F Bruner H. D and Pennes H H The direct spectrophorometric determination of the saturation of hemoglobin in the arterial blood of man Amer J med Sci 208 135 1949

Dreifus, L S Bender S., Goldberg H and Downing D F The electrocardiogram in atrial septal defect Dis Chest. 36 521 1959

DuBois, D and DuBois, E F A formula to estimate the approximate surface area if height and weight be known Arch intern. Med 17 863 1916

DuShane J W Weidman, W H Brandenburg, P O., and Kirklin J W Differentiation of inter atrial communications by clinical methods Ostium secundum, ostium primum, common atrium, and total anomalous pulmonary venous connection Circulation 21 363 1960

Efskind, L. Husom, O Mollestad, O and Storstein O Atrieseptumdefekter behandlet under aapen kardiotomi T norske Laegeforen. 79 1043 1959

Eisenberg. R., and Hultgren, N R Phonocardiographic features of atrial septal defect Circulation 20 490 1959

Ekelund L. G and Holmgren, A Central hemodynamics during exercise Circ Research 20 Suppl. 1 33 1967

Ellis F H Jr Brandenburg, R O and Swan, H J C Defect of the atrial septum in the elderly Report of successful surgical correction in five patients sixty years of age or older New Engl J Med. 262 219 1960

Ellis, F R. Greaves M and Hecht H H Congenital heart disease in old age Interauricular septal defect with mitral and tricuspid valvulitis Amer Heart J 40 154 1950

Feigenbaum H Campbell R. W Wunsch Ch. M and Steinmetz E. F Evaluation of the left ventricle in patients with mitral stenosis Circulation 34 462 1966

Feruglio G A. An intracardiac sound generator for the study of the transmission of heart murmurs in man. Amer Heart J 63 232 1962

Fisher J M Wilson W R and Theilen E O Recognition of congenital heart disease in the fifth to eighth decades of life Diagnostic criteria and natural history Circulation 25 821 1962

Fleming H A Differential lung function in atrial septal defect. Circulation 19 856 1959

Fouche R F., Beck, W and Schrire V The roentgenologic assessment of the degree of left to-right shunt in secundum type atrial septal defect, Amer J Roentgenol. 89 254 1963

Freeman, A R and Levine S A The clinical significance of the systolic murmur Ann. intern Med 6 1371, 1933

Frick M H Observations on hemodynamic factors and left heart performance in essential hypotension. Acta med scand 172 Suppl. 380 1962 (Thesis Helsinki)

Frick M H Punsar S and Somer T The spectrum of cardiac capacity in patients with nonbstructive congenital heart disease Amer J Cardiol. 17 20 1966

Friedman, C E Heart volume myocardial volume *and total capacity of the heart cavities in certain* chronic heart diseases Acta med scand 140 257 1951

Fry D L Noble F W and Mallos, A J An evaluation of modern pressure recording systems Circulat. Res 5 40 1957

Gault, J H Morrow A. G Gay W A., and Ross J Jr Atrial septal defect in patients over age of forty years Clinical and hemodynamic studies and effects of operation Circulation 37 261, 1968

Gay B B Jr and Franch R H Pulsation in the pulmonary arteries as observed with roentgenoscopic image amplification observations in patients having increased pulmonary blood flow Amer J Roentgenol. 85 1025 1961

Kubis, M Lukl, P., Ressl, J., and Vykydal, J Changes of the first heart sound in atrial septal defect. Atti soc ital. cardiol. XXVII congr 1966 vol. II communicazioni Roma 1967 p 5

Kurz, H., Slodki S J., and Luisada A. A. Left ventricular hemodynamics in mitral stenosis Amer J Cardiol. 19 183, 1967

Kuzman, W J., and Yuskis, A. S Atrial septal defect in the older patient simulating aquired val vular heart disease Amer J Cardiol 15 303 1965

Lagerlof H., and Werko L. Studies on the circulation of blood in man VI The pulmonary capillary venous pressure pulse in man. Scand J clin. Lab Invest. 1 147 1949

Lamb L. E. Electrocardiography and vectorcardiography Instrumentation, fundamentals and clinical applications. Saunders *Philadelphia & London*, 1965

Larsson H., and Kjellberg, S R Roentgenological heart volume determination with special regard to pulse rate and the position of the body Acta radiol. (Stockh.) 29 159 1948

Lauson, H D., Bloomfield R A. and Cournand A. Influence of respiration on circulation in man with special reference to pressures in right auricle right ventricle femoral artery and peripheral veins Amer J Med 1 315 1946

Leatham A Phonocardiography Brit med Bull. 8 333 1952

Leatham A. and Gray J Auscultatory and phonocardiographic signs of atrial septal defect. Brit. Heart J 18 193 1956

Leatham, A., and Vogelpoel, L. The early systolic sound in dilatation of the pulmonary artery Brit. Heart J 16 21 1954.

Lee Y C., and Scherlis L. Atrial septal defect. Electrocardiographic vectorcardiographic and cat heterization data Circulation 25 1024 1962

Lee Y C and Scherlis, L Some problems in the vectorcardiographic diagnosis of right ventricular hypertrophy p 142 In Proc Long Island Jewish Hosp Symp Vectorcardiography J Hoffman and R C Taymor (ed.) North Holland Publ. Co., Amsterdam, 1966

Lev M Paul M H and Cassels D E. Complete atrioventricular block associated with atrial septal defect of the fossa ovalis (secundum) type A histopatholocig study of the conduction systems Amer J Cardiol. 19 266 1967

Levin, A R. Spach, M. S., Boineau J P., Canent, R. V., Capp M P and Jewett, P H. Atrial pressure flow dynamics in atrial septal defects (secundum type) Circulation 37 476 1968

Levine H J., Neill, W A. Wagman, R. J Krasnow N., and Gorlin, R The effect of exercise on mean left ventricular ejection rate in man. J clin. Invest. 41 1050 1962.

Levinson, G E., Pacifico A D., and Frank, M. J.. Studies of total cardiopulmonary blood volume in normal human subjects at rest and during exercise Circulation 33 347 1966

Lewis, B M., Houssay H E. J., Haynes F W., and Dexter L. The dynamics of both right and left ventricles at rest and during exercise in patients with heart failure Circulation Res. 1 312 1953.

Lewis, F J., and Taufic, M. Closure of atrial septal defects with aid of hypothermia experimental accomplishment and report of one successful case Surgery 33 52, 1953

Liddle H V., Meyer B W., and Jones J C The results of surgical correction of atrial septal defect complicated by pulmonary hypertension. J thorac cardiovasc Surg 39 35 1960

Liljestrand, G., Lysholm, E. Nylin, G., and Zachrisson, C G Normal heart volume in man. Amer Heart J 17 406 1939

Limon, L R., Esclavissat, M., Puech, P., De la Cruz, M V., Rubio V Bouchard F., and Soni, J El cateterismo intracardiaco V La comunicacion interauricular Correlacion de los hallazgos hemodinamicos con los datos embriologicos, clinicos radiologicos y electrocardiograficos en 50 casos. Arch. Inst. Cardiol. Mex. 23 279 1953.

Lind J., and Wegelius, C Atrial septal defects in children. Circulation 7 819 1953

Linden, R. J., and Mitchell, J H Relation between left ventricular diastolic pressure and myocardial segment length and observations on the contribution of atrial systole Circulat. Res. 8 1092, 1960

Linko E., Kajas, S., Melartin, E. and Sysimetsä, E. Pienoisrontgen (RP)-joukkotutkimus sydanvikojen paljastajana. Duodecim (Helsinki) 79 374, 1963.

Lipman, B S., and Massie E. Clinical scalar electro cardiography Year Book Medical Publ Chicago 1965 Ed 5

Little R C., Oppdyke D F., and Havley J G Dynamics of experimental atrial septal defects Amer J Physiol. 158 241 1949

Logue R. B., Rogers, J V., and Gay B B., Jr Subtle roentgenographic signs of left heart failure Amer Heart J 65 464 1963

Loogen, F Schaub W., and Toker Y Spatergebnisse nach operativem Verschluss eines Vorhofseptumdefektes (Foramen secundum -Typ) Z Kreisl Forsch 50 1062 and 1138 1961

Lopez, J F., Linn, H. and Shaffer A B The apical first heart sound as an aid in the diagnosis of atrial septal defect Circulation 26 1296 1962.

Luchsenger P C., Seipp H. W., and Patel, D J Relationship of pulmonary artery wedge pressure to left atrial pressure in man. Circulat. Res 11 315 1962.

Luisada A A., and DiBartolo G High frequency phonocardiography Amer J C...

Luisada A A Slodki S J Metha S and Gupta P D Two mechanisms of split second heart sound in a patient with atrial septal defect. Amer J Cardiol. 18 788 1966

McGoon D C Swan H J C., Brandenburg R O Connolly D C and Kirklin, J W Atrial septal defect factors affecting the surgical mortality rate Circulation 19 195 1959

Macruz, R Perloff J K. and Case R B A method for the electrocardiographic recognition of atrial enlargement. Circulation 17 882 1958

Mahoney E. B Manning J A DeWees J A and Schwartz, S Clinical results of correction under hypothermia of atrial septal defects and pulmonary valvular stenosis. J thorac cardiovasc Surg 38 292 1959

Mark, H Natural history of atrial septal defect with criteria for selection for surgery Amer J Cardiol 12 66 1963

Markman, P Howitt, G and Wade E G Atrial septal defect in the middle-aged and elderly Quart. J Med N S 34 409 1965

Marshall, H W Helmholz, H. F Jr and Wood E A Physiologic consequences of congenital heart disease p 461 In Handbook of physiology W F Hamilton and P Dow (ed) Sect. 2 vol 1 American Physiol Soc., Washington D C 1962

Massie E and Walsh, T J Clinical vectorcardiography and electrocardiography The Year Book Publ. Chicago 1960

Massumi R and Tawakkol, A A Direct study of left atrial P waves Amer J Cardiol 20 331 1967

Milnor W R The elctrocardiogram and vectorcardiogram in right ventricular hypertrophy and right bundle branch block. Circulation 16 348 1957

Mirowski M Left atrial rhythm. Diagnostic criteria and differentiation from nodal arrhythmias Amer J Cardiol. 17 203 1966

Morris J J Estes E H Whalen, R E Thompson H K. and McIntosh, H D P wave analysis in valvular heart disease Circulation 29 242 1964

Morrow A G Oldham H N Callard, G M and Braunwald E. The assessment of operative results in congenital heart disease by intraoperative indicator dilution curves. Circulation 33 263 1966

Musshoff K., and Reindell H Zur Rontgenuntersuchung des Herzens in horisontaler und vertikaler Korperstellung. I Mitteilung der Einfluss der Korperstellung auf das Herzvolumen. II Mitteilung der Einfluss der Korperstellung auf die Herzform. Dtsch. med Wschr 81 1001 and 82 26 1957

Musshoff K Reindell, H and Klepzig H Stroke volume arterio venous difference cardiac output and physical working capacity and their relationship to heart volume Acta cardiol. (Brux) 14 427 1959

Mustard W T Atrial septal defects — the results of surgery p 171 In Congenital heart disease D P Morse (ed) Davis Philadelphia 1962.

Nahas G G Spectrophotometric determination of hemoglobin and oxyhemoglobin in whole hemolyzed blood Science 113 723 1951

Nakamura F F and Nadas A S Complete heart block in infants and children New Engl. J Med 270 1261 1964

Nerard M-A Contribution a letude de la communication interauriculaire Lyon 1958 (Thesis Lyon)

New York Heart Association the Criteria Committee Diseases of the heart and blood vessels Nomenclature and criteria for diagnosis Ed 6 Little Brown and Co Boston 1964

Nielsen, J S and Fabricius J The effect of exercise on the size of the shunt in patients with atrial septal defects. Acta med scand 183 91 1968

Nixon, P G F and Wooler G H Rapid left ventricular filling and stasis in mitral regurgitation. Brit. Heart J 23 161 1961

Nora J J McNamara D G and Fraser F C Hereditary factors in atrial septal defect. Circulation 35 448 1967

Novack P Segal, B Kasparian, H and Likoff W Atrial septal defect in patients over 40 Geriatrics 18 421 1963

Oakley C Taylor S Wilcken D., Hollman, A., Goodwin, J F., and Shillingford J Dye dilution curves in congenital heart disease Brit. Heart J 22 533 1960

Öhnell, R. F Pre Excitation, a Cardiac Abnormality Acta Med Scand. 117 Suppl. 152, 1944 (Thesis Stockholm)

Okada R., Glagov S and Lev M. Different effects of increased volume and increased pressure on endocardial structure in hearts with atrial septal defect. Amer Heart J 75 474, 1968

Oravetz J Wissner S Argano B and Luisada A A Dynamic analysis of heart sounds in right and left bundle branch blocks. Circulation 36 275 1967

Paloheimo J A v Essen R Klemola E Kaariainen L and Siltanen, P Subclinical cytomegalovirus infections and cytomegalovirus mononucleosis in connection with open heart surgery Amer J Cardiol. (in press) 1968

Paloheimo J A., and Halonen, P I A case of mononucleosis like syndrome after blood transfusion from a donor with asymptomatic mononucleosis J cardiovasc Surg. (Torino) 6 558 1965

Papp C Cardiac syncope in atrial septal defect. Brit. Heart J 20 9 1958

Patel D F Mason, D T Ross J Jr., and Braunwald E Harmonic analysis of pressure pulses obtained from the heart and great vessels of man. Amer Heart J 69 785 1965

Patten, B M Developmental defects at the foramen ovale Amer J Path. 14 135 1938

Perloff J K Caulfield W H and DeLeon, A C Peripheral pulmonary artery murmur of atrial septal defect. Brit. Heart J 29 411 1967

Perasalo O Halonen P I and Siltanen, P Endocardial cushion defect. Clinical and surgical considerations. Acta chir scand 128 592, 1964

Pernkopf E. Die Transposition der Venenmundungen im Bereiche des Herzvorhofes Entwicklungs geschichtliche Analyse dieser Erscheinung zugleich ein Beitrag zur Entwicklungsgeschichte des Herzens im Besonderen der Vorhofsabteilung, p 713 In Die Transposition der Venenmündungen II By A Kiss, H Partilla and E Pernkopf Virchows Arch path Anat 324 707 1954 (Quoted by Davidsen 1960)

Peter R. H., Morris, J J., and McIntosh, H D Relationship of fibrillatory waves and P waves in the electrocardiogram. Circulation 33 599 1966

Petersson, P O Atrial septal defect of secundum type A clinical study before and after operation with special reference to haemodynamic function Acta paediat. scand Suppl. 174 1967 (Thesis Uppsala)

Pietila, K. Jaaskelainen, J., Hakkila J., and Siltanen, P Diagnostic criteria for 70-mm RP in mass screening for heart disease (on preparation)

Popper R W., Knott, J M S Selzer A and Gerbode F Arrhythmias after cardiac surgery I Uncomplicated atrial septal defect. Amer Heart J 64 455 1962

Poppius, H Airway obstruction, distribution of inspired gas, and physiologic dead space in pulmonary tuberculosis Ann Acad Sci. fenn. A V 114 1965 (Thesis, Helsinki)

Porje I G Studies of the arterial pulse wave particularly in the aorta Acta physiol. scand. 13 Suppl. 42 1946

Pryor R. Woodwark, G M and Blount, S G Jr Electrocardiographic changes in atrial septal defects ostium secundum defect versus ostium primum (endocardial cushion) defect. Amer Heart J 58 689 1959

Pyorala K. Punsar S Siltanen, P and Lehtovirta, E Cardiovascular survey on policemen in Helsinki (Unpublished observations)

Rackley C E Hurlburt J C., Harris, C W Floyd W L and Orgain, E S Postoperative use of the hydrogen sensitive platinum tipped electrode Detection of residual left to right shunts Amer J Cardiol. 19 692 1967

Reeve R. Selzer A., Popper R W Leeds R F and Gerbode F Reversibility of pulmonary hypertension following cardiac surgery Circulation 34 Suppl 1 107 1966

Reeves J T Groves, R. F Filley G T and Blount, S G Jr Cardiac output of normal resting man. J appl. Physiol 16 276 1961

Rehder K. Kirklin, J W and Theye R A Physiologic studies following surgical correction of atrial septal defect and similar lesions Circulation 26 1302, 1962

Reindell, H., Doll, E Steim, H Bilger F Konig K. Gebhardt, W and Emmrich, J Das pre und postoperative Rontgenbild angeborener Herzfehler seine Bedeutung fur Diagnose Prognose und Pathophysiologie Mitteilung II Der Vorhofseptumdefekt. Arch Kreisl. Forsch 39 71 1962

Reinhold J Venous pulse in atrial septal defect a clinical sign Brit. med J I 695 1955

Reynolds, J L. The electrocardiographic recognition of left atrial enlargement in childhood Amer Heart J 74 179 1967

Richardson, D W., Wyso W M., Hecht, A M and Fitzpatrick, D P Value of continuous photo electric recording of dye curves in the estimation of cardiac output Circulation 20 1111 1959

Rodstein, M Zeman, F D and Gerber I E, Atrial septal defect in the aged Circulation 23 665 1961

Roesler H Interatrial septal defect. Arch intern. Med 54 339 1934

Rogers, H. M and Edwards, J E Incomplete division of the atrioventricular canal with patent interatrial foramen primum (persistent common atrioventricular ostium) Report of five cases and review of the litterature Amer Heart J 36 28 1948

Rohrer F Volumenbestimmung von Körperhohlen und Organen auf orthodiagraphischem Wege Fortschr Rontgenstr 24 285 1916

Rokitansky C Die Defecte der Scheidewande des Herzens pathologischanatomische Abhandlung Braumuller Wien, 1875

Rokseth, R Congenital heart disease in middle aged adults. Acta med scand 183 131 1968

Roman G T., Jr Walsh, T J., and Massie E. Right ventricular hypertrophy Correlation of electrocardiographic and anatomic findings. Amer J Cardiol. 7 481 1961

Ross, J., Jr Braunwald E., Mason, D T., Braunwald, N S and Morrow A G Interatrial communication and left atrial hypertension A cause of continuous murmur Circulation 28 853, 1963

Ross J Jr Gault, J H., Mason, D T., Linhart, J W and Braundwald E Left ventricular performance during muscular exercise in patients with and without cardiac dysfunction. Circulation 34 597 1966

Ross, J Jr Linhart, J W and Braunwald E. Effects of changing heart rate in man by electrical stimulation of the right atrium Studies at rest, during exercise and with isoproterenol Circulation 32 549 1965

Rossall R E. and Gunning A J Basal horizontal lines on chest radiographs Significance in heart disease Lancet I 604 1956

Routier D Brumlik, J and Malinsky A De l electrocardiogramme dans les cardiopathies congenitales Allongement de la duree de l onde rapide et bloc de la branche droite Arch. Mal. Cœur 33 40 1940

Rowe G G Castillo C A Maxwell, G M., Clifford J E. and Crumpton, C W Atrial septal defect and the mechanism of shunt Amer Heart J 61 369 1961

Rushmer R. F and Smith, O A Cardiac control Physiol. Rev 39 41, 1959

Saltzman, G F The conventional roentgenogram in the commonest congenital malformations of the heart and great vessels in adults and juveniles Acta radiol. (Stockh.) Suppl. 114 28 1954

Samet, Ph Bernstein, W H., Silverman L. and Kramer C Indicator dilution curves in atrial septal defect. Brit. Heart J 25 191 1963

Sanchez Cascos, A., and Deuchar D The P wave in atrial septal defect. Brit. Heart J 25 202 1963

Sano T Hellenstein, H K and Vayda E P vector loop in health and disease as studied by the technique of electrical dissection of the vactorcardiogram. Amer Heart J 53 854 1957

Sarajas H S Heart damage in dogs subjected to hypothermia with or without complicating cardiac operations Electrocardiographic and pathologico-anatomic observations with some data on circulatory and blood changes. Ann. Acad Sci fenn. A. V 86 1961 (Thesis Helsinki)

Sarnoff S J and Berglund, E Ventricular function I Starling's law of the heart studied by means of simultaneous right and left ventricular function curves in the dog Circulation 9 706 1954

Sarnoff S J Braunwald, E Welch, G H., Jr Case R B., and Stainsby W N Hemodynamic determinants of oxygen consumption of the heart with special referance to the tension time index Amer J Physiol. 192 148 1958

Sarnoff S J and Mitchell, J H The regulation of the performance of the heart. Amer J Med 30 747 1961

Seebat, L Voridis E Renais J and Lenegre J Etude du mecanisme des shunts dans les communications inter auriculaires interventriculaires et aorto pulmonaires Arch. Mal. Cœur 50 818 1957

Schmutzler H Mellerowicz, H and Carl, E Über die bedeutung des Sauerstoffpulses zur Beurteilung der Leistungsbreite bei Kranken mit angeborenen und erworbenen Herzfehlern. Z Kreisl.-Forsch. 49 445 1960

Schrire V Beck, W Vogelpoel, L Nellen, M and Barnard C N Atrial septal defect. Part I secundum and sinus venosus defects S Afr Med. J 37 727 1963

Scott. R. C The correlation between the electrocardiographic patterns of ventricular hypertrophy and the anatomic findings. Circulation 21 256 1960

Scott. R. C The electrocardiographic diagnosis of right ventricular hypertrophy in the adult. Heart Bull. 16 65 1967

Scott, R. C Seiwert, V J., Simon, D L and McGuire J Left ventricular hypertrophy Circulation 11 89 1955

Segall, H N A simple method for graphic description of cardiac auscultatory signs Amer Heart J 8 533 1933

Seldon, W A., Rubinstein, C and Fraser A A. The incidence of atrial septal defect in adults Brit. Heart J 24 557 1962

Sellers R D Ferlic R. M Sterns, L P and Lillehei C W Secundum type atrial septal defects Early and late results of surgical repair using extracorporeal circulation in 275 patients Surgery 59 155 1966

Shaffer A B Silber E N and Katz, L. N Observations on the interatrial pressure gradients in the man. Circulation 10 527 1954

Shah, P M Mori M MacCanon, D M and Luisada A A Hemodynamic correlates of the various components of the first heart sound Circulat Res 12 386 1963

Siltanen, P The cross sectional area of the aortic arch as estimated from ordinary postero-anterior chest radiograms (In preparation.)

Siltanen P Kaila E and Attila U Statistical analysis of the distribution of electrocardiographic mean vector planar orientation (In preparation.)

Siltanen, P., Merikallio E and Perasalo O Total body perfusion by utilizing dilution with prime containing plasma expander Ann. Chir Gynaec Fenn. 55 155 1966

Simonson, E. Differentiation between normal and abnormal in electrocardiography Mosby St Louis 1961

Sjostrand T Changes in the respiratory organ of workmen at an ore smelting works Acta med scand 128 Suppl. 196 687 1947

Sjostrand T Die pathologische Physiologie der Korrelationen zwischen Herz und Gefassystem Verh dtsch. Ges. Kreisl.-Forsch. 22 143 1956

Sjostrand T Exercise tests p 515 In Clinical physiology Pathophysiological basis and practical application T Sjostrand (ed) Sv Bokforl. Stockholm, 1967

Sokolow M. and Lyon T P The ventricular complex in right ventricular hypertrophy as obtained by unipolar precordial and limb leads Amer Heart J 38 273 1949

Somer T., and Frick, M H Declining incidence of rheumatic fever in the Finnish defense forces Military Med 130 807 1958

Somerville J Ostium primum defect Factors causing deterioration in the natural history Brit. Heart J 27 413 1965

Sommer L. S., and Voudoukis I. J Atrial septal defect in older age groups With especial reference to atypical clinical and electrocardiographic manifestations. Amer J Cardiol. 8 198 1961

Søndergaard, T Closure of atrial septal defects Acta chir scand 107 492 1954

Søndergaard T Cirkumclusion af atrieseptumdefekter Resultat af 101 operationer Nord Med 6: 680 1962

Soulie P Carlotti J., Joly F Acar J., and Forman, J Les communications inter auriculaires a propos de 81 cas Sem Hop (Paris) 35 609 1959

Spitz, H B The roentgen features of atrial septal defect. Heart Bull. 16 25 1967

Steiner R E. Radiological appearances of pulmonary vessels in pulmonary hypertension. Brit J Radiol. 31 188 1958

Storstein, O., and Efskind L Atrial septal defect. Clinical and hemodynamic findings and results of open heart surgery Acta chir scand 125 52, 1963

Strandell T Heart rate and work load at maximal working intensity in old men. Acta med scand 176 301 1964

Strano A Campa, P P., Condorelli M Dagianti G Filocamo A., Parrinello A and Tedeschi, A Fisiopatologia clinica delle pervieta del setto interatriale Atti XXVII congr soc ital. cardiol I 20 1967

Svanberg L. Influence of posture on the lung volumes, ventilation and circulation in normals A spirometric bronchospirometric investigation Scand J clin. Lab Invest 9 Suppl 25 1957 (Thesis Malmo)

Swan H J C Surgical closure of interauricular septal defects J Amer med Ass 151 792 1953

Swan, H J C., Kurtz, A B Davies, D H and Blount, S G Jr Atrial septal defect secundum J thorac Surg 37 52, 1959

Swan, H J C Burchell, H. B and Wood E. H The presence of venoarterial shunts in patients with interatrial communications Circulation 10 705 1954

Swan H J C., Kirklin, J W., Becu L M., and Wood E H Anomalous connection of right pulmonary veins to superior vena cava with interatrial communications Hemodynamic data in eight cases. Circulation 16 54 1957

Swan, H J C., Marshall, H W., and Wood E H The effect of exercise in the supine position on pulmonary vascular dynamics in patients with left-to-right shunts J clin. Invest 37 202, 1958

Tala, P., Halonen, P I Siltanen, P., and Merikallio F Cardiac surgery at the Clinic for Thoracic Surgery of the University Central Hospital, Helsinki, from 1915 to 1966 Ann. Chir Gynaec Fenn, 56 1, 1967

Tala P Poppius H., Siltanen P., and Heinonen A. Transfer factor for carbon monoxide before and after surgical correction of atrial septal defect. Ann. Chir Gynaec Fenn 57 135 1968

Taylor H. L., Buskirk E. and Henschel, A. Maximal oxygen intake as an objective measure of cardiorespiratory performance J Appl. Physiol. 8 73, 1955

Tibblin, G High blood p… A population study of m… med scand 18… Sur… Goteborg)

Tikoff G., Schmidt, A … H H Heart failure in atrial sep… J Med 39 533 19…

Toscano Barboza … Brandenb… Burchell, H B … cases with intrac… the atrioventricular canal … 1956

Tung K S K James T N Effler D B and McCormack L J Injury of the sinus node in open heart operations J thorac cardiovasc Surg 53 814 1967

Uhley H N., Leeds S E Simpson J J and Friedman M … of pulmonary … chronic pulmonary edema Circulat. Res … 1962

VanBogaert, A New concep… on the mechanism of the first heart sound Amer J Cardiol. 18 253 1966

Vierhout R R. and Vendrik A J H A hydr… pressure generator for testing the dyn… characteristic of catheters and manometers J Lab clin. Med 58 539 1961

Voegt, H Gerhard W and S… R … Verbesserung der F… durch Injektion des Indikators in die Vena anonyma Z Kreisl. Forsch. 51 345 1962

Wade O L C… index vs oxygen uptake during exercise in man, p … In Handbook of circulation Analysis and compilation P L Altman, D S Dittmer and R. M Grebe (ed.) Saunders, Philadelphia 1959

Wagstaffe W … Two cases of free communication between the auricles, by deficiency of the upper part of the septum auricularum. From cases aged 52 and 6 respectively No cyanosis. Tr path. Soc Lond 19 96 1868 (quot. by Davison 1960)

Wahlund H Determination of the physical working capacity Acta med scand 132 Suppl 215 1948 (Thesis, Stockholm)

Wakai, C S., and Edwards, J E. Developmental and pathologic considerations in persistent common atrioventricular canal Proc Mayo Clin. 31 487 1956

Walker W J., Matungly T W., Pollock, B E., Carmichael D B Inmon T W., and Forrester R H Electrocardiographic and hemodynamic correlation in atrial septal defect. Amer Heart J 52 547 1956

Weidman, W H Swan, H J C., DuShane J W., and Wood, E. H. A hemodynamic study of atrial septal defect and associated anomalies involving the atrial septum. J Lab clin. Med 50 165 1957

Wennevold A. The diastolic murmur of atrial septal defects as detected by intracardiac phonocardiography Circulation 34 132, 1966

Wennevold, A The Valsalva maneuver after operation for atrial septal defect Acta med scand 182 575 1967

Wilson, R. N Rosenbaum, F F., and Johnston, F D Interpretation of the ventricular complex of the electrocardiogram Advanc. intern. Med Interscience Publ., New York 2 1, 1947

Winchell, P., and Bashour F Some physiological features of atrial septal defect. Amer J Cardiol 2 687 1958

Winters, L. W., Jr., Cortes, F., McDonough, M. Tyson, R. R. Baier H., Gimenez, J and Davila J C Venoarterial shunting from inferior vena cava to left atrium in atrial septal defects with normal right heart pressures Report of two cases. Amer J Cardiol. 19 293 1967

Wolf P S., Vogel, J H K Pryor R and Blount S G., Jr Atrial septal defect in patients over 45 years of age Merits of surgical versus medical therapy Brit. Heart J 30 115 1968

Wood, P Congenital heart disease Brit. med J II 639 653 1950

Wood, P Diseases of the heart and circulation Ed. 2. Eyre & Spottiswoode London 1957

Wood P The Eisenmenger syndrome or pulmonary hypertension with reversed central shunt. Brit med J II. 701 755 1958

Wood, P Atrial septal defect, p 49 In. Congenital heart disease D P Morse (ed) Davis, Philadelphia 1962.

Woolf C R. Pulmonary function in adults with intracardiac septal defect. Circulation 27 261, 1963

Woolf C R Paul, W and Gunton R W The diagnostic use of an ear oximeter in congenital heart disease Brit. Heart J 20 311 1958

Yu, P N., Murphy G W., Schreiner B F., and James, D H Distensibility characteristics of the human pulmonary vascular bed Study of the pressure volume response to exercise in patients with and without heart disease Circulation 35 710 1967

Zaver A G and Nadas, A S Atrial septal defect — secundum type Circulation 32 Suppl. 3 24 1965

Woolf C R Pulmonary function in adults with intracardiac septal defect Circulation 27 261 1963

Woolf C R Paul, W and Gunton R W The diagnostic use of an ear oximeter in congenital heart disease Brit. Heart J 20 311 1958

Yu, P N., Murphy G W Schreiner B F and James D H. Distensibility characteristics of the human pulmonary vascular bed Study of the pressure volume response to exercise in patients with and without heart disease Circulation 35 710 1967

Zaver A G and Nadas A S Atrial septal defect — secundum type Circulation 32 Suppl. 3 24, 1965

Zuckerman H. S Zuckerman, G H Mammen, R. E and Wassermil M Atrial septal defect Familial occurrence in four generations of one family Amer J Cardiol. 9 515 1962

APPENDIX

Normal values in right heart catheterization

Findings at right heart catheterization in 17 healthy subjects (4 men and 13 women) at rest in supine position in 5 of them also during supine exercise with bicycle ergometer and 200—400 kpm/min load The same methods were employed in the test procedure and in analysing the data as in the present study on patients with ASD

		N	$\bar{x}$	SD	Range	Unit
V_{o_2}		17	207 5	31 8	173—270	ml/min
V_o /BSA		17	134 3	19 0	109—177	ml/min
AVD		17	4 04	0 70	2 5—5 6	vol %
HR		17	75 7	15 9	47—108	beats/min
Q		17	5 35	1 34	4 1—8 6	l/min
Q		17	3 47	0 80	2 7—5 2	l/min
SV		17	72 8	18 6	45—102	ml
SVI		17	46 9	10 9	32—67	ml
$MSER_{RV}$		17	134 5	28 7	90—185	ml/sec
$MSER_{LV}$		14	147 1	9 1	94—191	ml/sec
SWI_{RV}		17	10 4	2 6	5—14	g m
SWI_{LV}		14	60 4	18 6	33—86	g m
PTI_{RV}		17	6 8	1 2	4—9	mmHg sec
PTI_{LV}		14	33 0	6 4	24—46	mmHg sec
R_p		17	97	36	34—163	dyne sec/cm[5]
R		17	150	54	51—241	dyne sec/cm[5]
R		14	1407	354	855—2057	dyne sec/cm[5]
R		14	2137	397	1465—3044	dyne sec/cm[5]
$D\ 1_{RV}$		17	19 36	14 60	6 3—59 7	ml/mmHg
$D\text{-}1_{LV}$		17	8 16	3 92	3 6—16 1	ml/mmHg
$D\ 2_R$		17	13 44	6 85	5 9—29 7	ml/mmHg
$D\ 2_{LV}$		17	10 38	7 66	3 8—32 9	ml/mmHg
$D\ 3_{RV}$		17	0 92	0 66	0 2—2 4	
$D\ 3_{LV}$		17	1 28	0 75	0 7—3 7	
P_R	(a-wave)	17	4 6	1 6	2—6	mmHg
	(v wave)	16	3 7	1 7	2—7	mmHg
	(mean)	17	2 8	1 4	2—5	mmHg
P_{RV}	(s)	17	25 2	6 1	13—36	mmHg
	(ed)	17	3 3	1 7	1—6	mmHg
P_{I}	(s)	17	22 0	4 7	12—29	mmHg
	(d)	17	8 0	2 8	3—13	mmHg
	(mean)	17	13 1	2 8	8—18	mmHg
P_w	(a wave)	15	8 4	2 9	2—12	mmHg
	(v wave)	15	9 3	3 4	3—14	mmHg
	(mean)	17	6 7	2 2	3—10	mmHg
P	(s)	14	124 9	17 9	98—160	mmHg
	(d)	14	74 0	13 6	54—96	mmHg
	(mean)	14	89 7	14 7	73—115	mmHg
Exercise						
EF		5	1176 2	312 8	679—1487	
SV % change		5	+43 6	29 1	13—80	
$MSER_{LV}$ % change		5	+74 2	52 9	24—160	
$\bar{P}_w$ change		5	+ 5 4	2 9	2—9	mmHg

GLOSSARY OF SYMBOLS

Symbols of quantities, and abbreviations

- *A* = mean electrical axis in degrees ( )
- *AVD* = arteriovenous oxygen difference in vol %
- *BSA* = body surface area in m^2
- *BW* = body weight in kg
- *C* = concentration of gas (e g O) in blood phase in vol %
- *D-1 2 3* = indices related to ventricular compliance *D-1* and *D 2* in ml/mmHg
- *DFP* = diastolic filling period in sec/beat
- *EF* = exercise factor
- *FC* = functional class as rated according to New York Heart Association (N Y H A) recommendations
- $FEV_{1.0}$ = forced expiratory volume in per cent of actual *VC*
- *HR* = heart rate in beats/min
- *HV* = heart volume in ml
- *MSER* = effective mean systolic ejection rate in ml/sec
- *P* = blood pressure in mmHg
- *PTI* = pressure-time index per beat in mmHg sec
- PWC_{HR} = physical working capacity at fixed heart rate in kpm/min
- $\dot{Q}$ = volume flow of blood per unit time in l/min
- *R* = vascular resistance in dynes sec cm^{-5}
- *S* = per cent saturation of haemoglobin with oxygen
- *SEP* = systolic ejection period in sec/beat
- *SV* = stroke volume in ml
- *SVI* = stroke volume index in ml
- *SWI* = stroke work index in g m
- *V* = gas volume per unit time (e g oxygen uptake V_{o_2}), in ml/min
- *VC* = vital capacity in litres

Symbols mainly occurring as subindices

- *BTPS* = body temperature and pressure saturated with water vapour
- *IVC* = inferior vena cava
- *LA* = left atrium
- *LV* = left ventricle
- *PA* = pulmonary artery
- *RA* = right atrium
- *RV* = right ventricle
- *SVC* = superior vena cava
- *STPD* = 0 *760 mmHg dry*
- *W* = pulmonary artery wedge position
- *a* = arterial (pressure blood)
- d = diastolic (pressure)
- ed = end-diastolic
- *i* = relative to *BSA* i e an index
- *p* = *pulmonary circulatory (e g* Q_p)
- *s* = as secondary symbol systemic circulatory (e g *Q*) as tertiary symbol systolic (e g P_{ps})
- *v* = venous (blood pressure)
- ‾ = bar above any symbol indicates a mean value (e g $\bar{v}$ = mixed venous $\bar{P}$ = mean pressure)

STATISTICAL TREATMENT

In various connections of the present study the results which were obtained in the series and in its different subgroups were analyzed by methods of statistical mathematics This was done employing the following generally known formulae or equivalent ones, in which x (and y) indicate individual (pairs of) observations and N their number, with subindices indicating e g different populations etc

Mean $\bar{x} = \sum x/N$

Standard deviation $SD = \sqrt{(x-\bar{x})/(N-1)} = s$ similarly s_y

Confidence interval of mean (at level P)

$$x = \bar{x} \pm s \quad t_{F, P}/\sqrt{N} \qquad F \text{ (Freedoms)} = N-1$$

Covariance of x and y $s_{xy} = (x-\bar{x})(y-\bar{y})/(N-1)$

Correlation coefficient $r = s_{xy}/s_x\ s_y$

Regression coefficient $b_{yx} = s_{xy}/s^2$

t test for two means

$$t = \sqrt{\frac{N_1 N_2 (N_1 + N_2 - 2)}{(N_1 + N_2)(N_1 s_x^2 + N_2 s_y^2)}} \quad |\bar{x}_1 - \bar{x}_2|$$

Freedoms $N_1 + N_2 - 2$

t test for difference of correlation coefficient from zero

$$t = \sqrt{\frac{r^2}{1-r}(N-2)} \qquad \text{Freedoms } N-2$$

t test for difference of regression coefficient from hypothetical value (b)

$$t = (b_{yx} - b) \quad \frac{s_x}{s_y}\sqrt{\frac{N-2}{1-r^2}} \quad \text{Freedoms } N-2$$

95 % tolerance limits for the regression of y on x

$$Y = \bar{y} + b_y \ (x-\bar{x}) \pm t_{0.95}\ s_T \qquad \text{Freedoms } N-2$$

$$s_T = \frac{s_y}{s}\sqrt{\frac{N-1}{1-r^2}}\sqrt{(1 + 1/N)(N-1)\ s_x^2 + (x-\bar{x})^2}$$

Standard error of estimate $s_{y\,x} = s_y \sqrt{1-r^2}\sqrt{\frac{N-1}{N-2}}$

Statistically significant differences were considered to exist on the strength of the t test as follows

When $P < 0.001$ statistically highly significant (***)
$P < 0.01$ statistically significant (**)
$P < 0.05$ statistically almost significant (*)

A special method of evaluation was employed in respect of the mean frontal axis in the electrocardiogram

Each mean frontal vector was treated as if its amplitude were unity, that is the sets of vectors were analyzed according to the distribution of their intersections with the unit radius circle In order to avoid the ambiguity of ± 360 degrees the phase angles were averaged by determining the coordinates of the centroid of the configuration of such points of intersection ξ and η

$$\xi = \Sigma \cos \Phi/N \quad \eta = \Sigma \sin\Phi/N$$

and from these the orientation of the corresponding vector

$$\tan \overline{\Phi} = \eta/\xi$$

the Φ and $\overline{\Phi}$ values being taken with reference to an arbitrary zero direction

As a usable approximation of the standard deviation of Φ the following value was used (Siltanen et al in preparation)

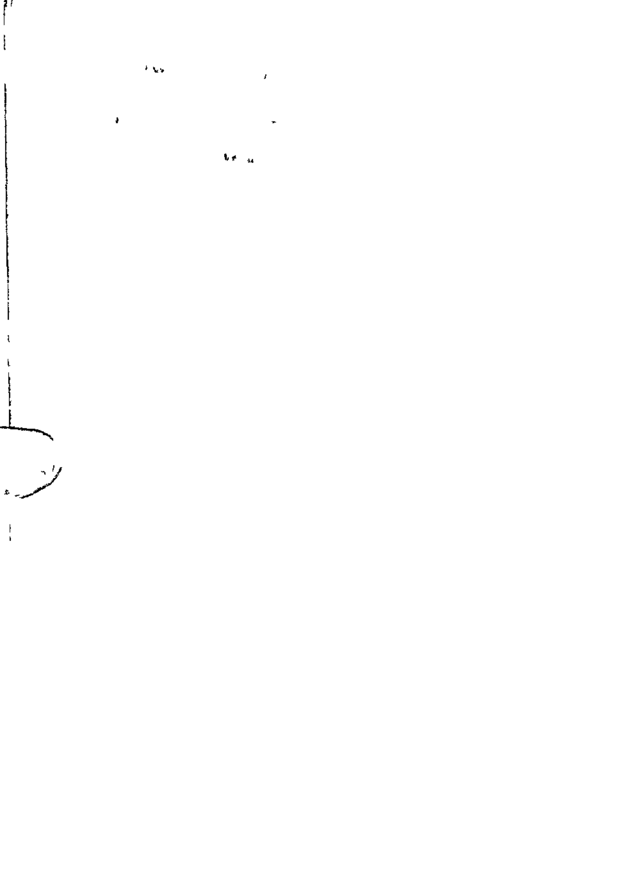
$$S.D._{\Phi} = 80 \sqrt{1 - \sqrt{\xi^2 + \eta^2}}$$

Acta Medica Scandinavica

Supplementum 498

Überlebenszeit und Abbau menschlicher Thrombozyten

von Dr. W Bleifeld



Überlebenszeit und Abbau menschlicher Thrombozyten

VON PRIV DOZ DR MED WALTER BLEIFELD

INHALTSVERZEICHNIS

Seit der Entdeckung der Thrombozyten durch DONNÉ im Jahre 1842 (62) und ihrer ersten Benennung als Blutplattchen" durch BIZZOZERO (30) im Jahre 1882 sind betrachtliche Kenntnisse uber Ursprung (189), Struktur (34) Stoffwechsel (25 28) Funktion (119) und Antigenitat (174 177 178) gewonnen worden Diese Erkenntnisse gewahren allerdings nur teilweise Einblick in die Ätiologie und die Pathogenese von thrombozytaren Erkrankungen So sind Blutplattchenzahl das Verhalten des Knochenmarks und gerinnungsphysiologische sowie serologische Befunde zwar wesentliche aber keineswegs immer den Mechanismus einer Thrombozytenerkrankung klarende Daten Dem eingangs erwähnten umfangreichen Wissen uber die Blutplättchen steht gegenüber daß die Kenntnis uber Dauer des Verbleibs und das Verhalten der Thrombozyten im Kreislauf erst in den letzten Jahren wesentlich erweitert worden ist Was schließlich den Abbauort der Thrombozyten unter normalen und pathologischen Bedingungen angeht so erklarte C A FINCH noch 1960 auf dem International Symposium on Blood platelets im Henry Ford Hospital in Dretoit I have no comment on platelet destruction since we haven t any evidence bearing on this (1)

Durch die Anwendung radioaktiver Isotope ist hier eine entscheidende Wandlung eingetreten

II KRITISCHE ÜBERSICHT

ÜBER METHODEN ZUR BESTIMMUNG DER THROMBOZYTENLEBENSDAUER

An Versuchen die Thrombozytenlebensdauer festzustellen hat es nicht gefehlt Die anfänglichen Studien die Überlebenszeit von menschlichen Thrombozyten durch Übertragung auf bestrahlte, thrombozytopenische Tiere zu bestimmen konnen hier außer acht gelassen werden, da sie verstandlicherweise zu keinen vergleichbaren Ergebnissen führten

Grundsätzlich bestehen folgende Möglichkeiten, die Lebensdauer von Blutplättchen zu untersuchen

1 Unterdruckung der Thrombozytenproduktion in den Megakaryozyten und Beobachtung der Plattchenzahl in den folgenden Tagen
2 Schnelle Zerstorung oder Beseitigung aller Thrombozyten aus dem Kreislauf und Untersuchung ihrer Regeneration aus den ungeschadigten Vorstufen
3 Übertragung ungeschadigter lebender Thrombozyten auf gesunde oder kranke Personen Besteht beim Empfänger eine starke Thrombozytopenie so ist eine spezielle Markierung der Blutplattchen nicht erforderlich und die Bestimmung der Überlebenszeit kann durch einfache Zahlung der Thrombozyten angenahert erfolgen Ist aber eine Vermehrung oder eine nur geringe Verminderung der Thrombozyten vorhanden so mussen die ubertragenen Blutplattchen an besonderen morphologischen funktionellen oder antigenen Eigenschaften im Empfänger erkennbar sein, damit eine verlassliche Ermittlung der Lebensdauer moglich ist
4 Markierung von Thrombozyten mit radioaktiven Substanzen
Eine solche Markierung mit radioaktiven Isotopen kann prinzipiell einmal dadurch geschehen, daß bestimmte radioaktive Stoffe einer Person injiziert werden. So findet in vivo" entweder eine unmittelbare Aufnahme des im Plasma befindlichen Isotops in die Thrombozyten statt oder das Isotop bindet sich an die Megakaryozyten und gelangt so uber den Megakaryozytenstoffwechsel in die Thrombozyten Der Kurvenverlauf der Radioaktivitat ist bei dem letzteren Verfahren unterschiedlich je nachdem, ob nur eine einzige Isotopendosis verabreicht wird oder durch wiederholte Applikation ein radioaktiver Spiegel aufrecht erhalten wird Andererseits konnen Plattchen auch aus dem Blut isoliert und dann ' in vitro" mit einem geeigneten Isotop gekoppelt und einer anderen oder derselben Person reinfundiert werden Der Verlauf der im Empfängerkreislauf festgestellten thrombozytat gebundenen Radioaktivitat gibt Aufschluß uber die Lebensdauer

Dementsprechend unterscheiden wir bei der Überlebenszeitbestimmung von Blutplattchen mit Hilfe radioaktiver Substanzen zwischen

a in vivo –Verfahren
  aa Durch unmittelbare Bindung des Isotops an die Blutplattchen.
  ab Durch Markierung der Thrombozytenvorstufen
    Nach einmaliger Injektion des Isotops
    Nach wiederholter Injektion
b in vitro –Verfahren

Zu 1

Tierexperimentelle Untersuchungen mittels hoher unter Umstanden letaler Dosen ionisierender Strahlen an Mausen Ratten, Katzen und Hunden (35 97 109 113 158 164 163 181 198) ließen die Überlebenszeit von Blutplattchen auf 3 bis 4 Tage bei Mäusen (109) und 8 Tage bei Hunden (181 198) festlegen Wie diese Untersuchungen scheidet auch die Anwendung chemischer Substanzen, insbesondere diejenige von Zytostatika (26 96) bei Menschen zu diagnostischen Untersuchungen aus begreiflichen Grunden aus

Dennoch ist auf diese Weise auch bei Menschen Aufschluß uber die Thrombozytenlebensdauer gewonnen worden denn zufallige Beobachtungen bei todlichen Strahlenunfallen (87 120) und nach Atombombenabwurfen (116) haben gezeigt daß nach einem Intervall von 1 bis 2 Tagen ein rapider Abfall der Blutplattchen auf extrem niedrige Werte erfolgt die nach 8 bis 9 Tagen erreicht werden Ähnliche Verlaufe sind nach hohen Dosen von Stickstofflost zu beobachten (126) Auf die Bedeutung dieser Befunde fur die Bewertung der eigenen Untersuchungen wird noch eingegangen werden

Zu 2

Akute Thrombozytopenien durch rasche Entfernung der Blutplattchen aus dem Kreislauf konnen tierexperimentell leicht durch Thromboplastin– und Fibrininnjektionen (10) durch Austauschtransfusion mit defibrinierem Blut (121) durch Plasmapherese (47) infolge Endotoxin–Injektion (172) Transfusion von *Antisera* (175 176 179) *sowie von* Plasma an idiopathischer Thrombozytopenie (ITP) Erkrankter (179) erzeugt werden Aus der anschließenden Regeneration bis zu Normalwerten sind Ruckschlusse auf die Lebensdauer der Thrombozyten gezogen worden Beim Menschen sind solche Verhaltnisse dann zu beobachten wenn die Thrombozyten durch Iso– oder Drogenimmunisierung (4 117) durch Transfusion von ITP–Plasma (85) oder bei akuten Defibrinierungssyndromen rasch zerstort werden Dabei zeigt sich grundsatzlich immer das gleiche Bild Nach einem mehr oder weniger langen Intervall erniedrigter Thrombozytenwerte erfolgt innerhalb 3 bis 5 Tagen ein Anstieg der Thrombozyten auf Normalwerte Es ist klar daß die hierdurch gewonnenen Befunde von 3 bis 5 Tagen beim Menschen nur als Minimalwerte anzusehen sind Sie halten daruber [illegible] auch insofern einer kritischen Betrachtung nicht stand als mindestens bei einem Teil der Untersuchungen Grund zur Annahme einer gleichzeitigen Megakaryozytenschadigung besteht (29) Daneben muß beachtet werden daß es auch Thrombozytensturze gibt bei denen die Blutplattchen nach wenigen Stunden wieder lebensfahig und funktionell nor

mal in den Kreislauf eintreten (8) wie insbesondere mit Radioisotopen durchgefuhrte Untersuchungen und solche mit Aethylendiamintetraessigsaure (EDTA) ungerinnbar gemachtem Blut gezeigt haben (170) Mit den genannten Untersuchungen lassen sich daher weder tierexperimentell noch beim Menschen verlassliche Befunde uber die Lebenszeit von Blutplattchen erheben Allerdings haben diese Studien ergeben daß die tagliche Regenerationsrate des Knochenmarks etwa 1/5 der gesamten Thrombozytenmasse betragt (22 63)

Zu 3

Eine weitere Moglichkeit Aufschluß uber die Lebenszeit der Blutplattchen zu gewinnen wurde in dem Verhalten der Plattchenzahl der Blutungs– und Gerinnungszeit sowie klinischer Besserung nach wegen hammorhagischer Diathese durchgefuhrter Transfusionen von Blut plattchenreichem Plasma und *Plasmakonzentraten gesehen* (25 72 106) *So* berichtete erstmals DUKE im Jahre 1910 uber eine Erhohung der Blutplattchenzahl fur die Dauer von 3 bis 4 Tagen und Verminderung der Blutungsneigung nach einer Direkttransfusion fur die entsprechende Zeit (64) Einen ahnlichen Befund konnten MINOT und LEE 1916 erheben (129) Aufgrund morphologischer Differenzierung (kongenital abnorme Blutplattchen) und durch Bestimmung der Plattchenzahl gelang es HIRSCH und Mitarbeitern im Jahre 1950 eine Lebensdauer von 5 Tagen bei einem Patienten zu ermitteln Es liegt auf der Hand daß es sich hierbei um einen seltenen Einzelbefund handelt dem keine generelle methodische Bedeutung zukommt Eine Identifizierung der Blutplattchen aufgrund ihrer Antigenstruktur in einem Empfangerkreislauf ahnlich wie bei der anfänglich fur die Bestimmung der Erythrozytenlebensdauer durchgefuhrten Methode von ASHBY (13) ist bisher nicht moglich

Insbesondere die Bestimmung der Plattchenzahl blieb fur die in der Folgezeit auf diese Weise durchgefuhrten Studien Kriterium fur die Überlebenszeit fur die angenaherte Werte bei verschiedenen Erkrankungen des Men

II KRITISCHE ÜBERSICHT

ÜBER METHODEN ZUR BESTIMMUNG DER THROMBOZYTENLEBENSDAUER

An Versuchen, die Thrombozytenlebensdauer festzustellen, hat es nicht gefehlt Die anfänglichen Studien, die Überlebenszeit von menschlichen Thrombozyten durch Übertragung auf bestrahlte, thrombozytopenische Tiere zu bestimmen, konnen hier außer acht gelassen werden da sie verstandlicherweise zu keinen vergleichbaren Ergebnissen fuhrten

Grundsatzlich bestehen folgende Moglichkeiten die Lebensdauer von Blutplattchen zu untersuchen

1 Unterdruckung der Thrombozytenproduktion in den Megakaryozyten und Beobachtung der Plattchenzahl in den folgenden Tagen
2 Schnelle Zerstorung oder Beseitigung aller Thrombozyten aus dem Kreislauf und Untersuchung ihrer Regeneration aus den ungeschädigten Vorstufen
3 Übertragung ungeschadigter lebender Thrombozyten auf gesunde oder kranke Personen Besteht beim Empfanger eine starke Thrombozytopenie so ist eine spezielle Markierung der Blutplattchen nicht erforderlich und die Bestimmung der Überlebenszeit kann durch einfache Zahlung der Thrombozyten angenahert erfolgen Ist aber eine Vermehrung oder eine nur geringe Verminderung der Thrombozyten vorhanden so mussen die ubertragenen Blutplattchen an besonderen morphologischen funktionellen oder antigenen Eigenschaften im Empfänger erkennbar sein damit eine verlassliche Ermittlung der Lebensdauer moglich ist
4 Markierung von Thrombozyten mit radioaktiven Substanzen
Eine solche Markierung mit radioaktiven Isotopen kann prinzipiell einmal dadurch geschehen, daß bestimmte radioaktive Stoffe einer Person injiziert werden. So findet "in vivo" entweder eine unmittelbare Aufnahme des im Plasma befindlichen Isotops in die Thrombozyten statt oder das Isotop bindet sich an die Megakaryozyten und gelangt so uber den Megakaryozytenstoffwechsel in die Thrombozyten Der Kurvenverlauf der Radioaktivitat ist bei dem letzteren Verfahren unterschiedlich, je nachdem ob nur eine einzige Isotopendosis verabreicht wird oder durch wiederholte Applikation ein radioaktiver Spiegel aufrechterhalten wird Andererseits konnen Plattchen auch aus dem Blut isoliert und dann in vitro" mit einem geeigneten Isotop gekoppelt und einer anderen oder derselben Person reinfundiert werden Der Verlauf der im Empfängerkreislauf festgestellten thrombozytat gebundenen Radioaktivität gibt Aufschluß uber die Lebensdauer

Dementsprechend unterscheiden wir bei der Überlebenszeitbestimmung von Blutplattchen mit Hilfe radioaktiver Substanzen zwischen

a ' in vivo –Verfahren
 aa Durch unmittelbare Bindung des Isotops an die Blutplattchen.
 ab Durch Markierung der Thrombozytenvorstufen
  Nach einmaliger Injektion des Isotops
  Nach wiederholter Injektion
b in vitro –Verfahren

Zu 1

Tierexperimentelle Untersuchungen mittels hoher, unter Umständen letaler Dosen ionisierender Strahlen an Mausen Ratten Katzen und Hunden (35 97 109 113 158 164 163 181 198) ließen die Überlebenszeit von Blutplattchen auf 3 bis 4 Tage bei Mäusen (109) und 8 Tage bei Hunden (181 198) festlegen Wie diese Untersuchungen scheidet auch die Anwendung chemischer Substanzen, insbesondere diejenige von Zytostatika (26 96) bei Menschen zu diagnostischen Untersuchungen aus begreiflichen Grunden aus

Dennoch ist auf diese Weise auch bei Menschen Aufschluß uber die Thrombozytenlebensdauer gewonnen worden, denn zufällige Beobachtungen bei todlichen Strahlenunfallen (87,120) und nach Atombombenabwurfen (116) haben gezeigt daß nach einem Intervall von 1 bis 2 Tagen ein rapider Abfall der Blutplattchen auf extrem niedrige Werte erfolgt die nach 8 bis 9 Tagen erreicht werden Ähnliche Verlaufe sind nach hohen Dosen von Stickstofflost zu beobachten (126) Auf die Bedeutung dieser Befunde für die Bewertung der eigenen Untersuchungen wird noch eingegangen werden

Zu 2

Akute Thrombozytopenien durch rasche Entfernung der Blutplattchen aus dem Kreislauf konnen tierexperimentell leicht durch Thromboplastin– und Fibrininjektionen (10) durch Austauschtransfusion mit defibriniertem Blut (121), durch Plasmapherese (47), infolge Endotoxin–Injektion (172) Transfusion von Antisera (175,176 179) sowie von Plasma an idiopathischer Thrombozytopenie (ITP) Erkrankter (179) erzeugt werden Aus der anschließenden Regeneration bis zu Normalwerten sind Ruckschlusse auf die Lebensdauer der Thrombozyten gezogen worden Beim Menschen sind solche Verhaltnisse dann zu beobachten, wenn die Thrombozyten durch Iso– oder Drogenimmunisierung (4 117) durch Transfusion von ITP–Plasma (85) oder bei akuten Defibrinierungssyndromen rasch zerstort werden Dabei zeigt sich grundsatzlich immer das gleiche Bild Nach einem mehr oder weniger langen Intervall erniedrigter Thrombozytenwerte erfolgt innerhalb 3 bis 5 Tagen ein Anstieg der Thrombozyten auf Normalwerte Es ist klar, daß die hierdurch gewonnenen Befunde von 3 bis 5 Tagen beim Menschen nur als Minimalwerte anzusehen sind Sie halten daruberhinaus auch insofern einer kritischen Betrachtung nicht stand als mindestens bei einem Teil der Untersuchungen Grund zur Annahme einer gleichzeitigen Megakaryozytenschadigung besteht (29) Daneben muß beachtet werden daß es auch Thrombozytensturze gibt bei denen die Blutplattchen nach wenigen Stunden wieder lebensfahig und funktionell normal in den Kreislauf eintreten (8) wie insbesondere mit Radioisotopen durchgeführte Untersuchungen und solche mit Aethylendiamintetraessigsaure (EDTA) ungerinnbar gemachtem Blut gezeigt haben (170) Mit den genannten Untersuchungen lassen sich daher weder tierexperimentell noch beim Menschen verlassliche Befunde uber die Lebenszeit von Blutplattchen erheben Allerdings haben diese Studien ergeben daß die tagliche Regenerationsrate des Knochenmarks etwa 1/5 der gesamten Thrombozytenmasse betragt (22 63)

Zu 3

Eine weitere Moglichkeit, Aufschluß uber die Lebenszeit der Blutplattchen zu gewinnen wurde in dem Verhalten der Plättchenzahl der Blutungs– und Gerinnungszeit sowie klinischer Besserung nach wegen hammorhagischer Diathese durchgeführter Transfusionen von Blut, plattchenreichem Plasma und Plasmakonzentraten gesehen (25 72 106) So berichtete erstmals DUKE im Jahre 1910 uber eine Erhohung der Blutplattchenzahl für die Dauer von 3 bis 4 Tagen und Verminderung der Blutungsneigung nach einer Direkttransfusion fur die entsprechende Zeit (64) Einen ahnlichen Befund konnten MINOT und LEE 1916 erheben (129) Aufgrund morphologischer Differenzierung (kongenital abnorme Blutplattchen) und durch Bestimmung der Plattchenzahl gelang es HIRSCH und Mitarbeitern im Jahre 1950, eine Lebensdauer von 5 Tagen bei einem Patienten zu ermitteln Es liegt auf der Hand daß es sich hierbei um einen seltenen Einzelbefund handelt dem keine generelle methodische Bedeutung zukommt Eine Identifizierung der Blutplattchen aufgrund ihrer Antigenstruktur in einem Empfängerkreislauf ahnlich wie bei der anfänglich für die Bestimmung der Erythrozytenlebensdauer durchgeführten Methode von ASHBY (13) ist bisher nicht moglich

Insbesondere die Bestimmung der Plattchenzahl blieb für die in der Folgezeit auf diese Weise durchgeführten Studien Kriterium für die Überlebenszeit für die angenaherte Werte bei verschiedenen Erkrankungen des Men-

Jahr	Autor	Isotop	HWZ *	Spezies	Lebensdauer	Abbauort	Technik	Bemerkungen
1952	JULLIARD u Mitarb (1o2)	P^{32}	14 Tg	Mensch	2 Std	Milz Leber Lunge (topisch bestimmt)	in itro	Auftreten von schweren Thrombo yten schädigungen bei der M rkierung
1953	MUELLER (133)	P^{32}	14 Tg	K ninchen	2 St	Milz Leber Lunge Kno chenm (autop bestimmt)	in itro	Bei der M rkierung tr ten Schädigun gen der Thrombozyten auf
1954	ODELL u Mitarb (149)	C^{14}	576o J	Ratte	3 4 Tg		in vi o	A ft eten von Thr mbozytenschädigun gen bei der M rkierung L nge HWZ
1954	ODELL u Mitarb (152)	C^{14} S^{35}(Sulf)	576o ! 87 1 Tg	R tte	5 6 Tg		in vivo	Lange HWZ Kei e Oberfl Aktiv meßb Lange HWZ Hohe Strahle bel
1954	MORGAN u Mitarb (167)	J^{131}	8 Tg	Kaninchen	1 2 Std		in vitro	Auftreten o schweren Thrombozyten chädigungen bei der Mark erung
1954	ROBERTSON u Mitarb (169)	$^{51}Cr\ Cl_3$	27 8 Tg	Ratte	2 Tg		in vitro	
1955	ODELL u Mitarb (153)	C^{14}	576o J	Ratte	1 3 Tg		in itro	Lange HWZ
1955	DESAI u Mitarb (58)	P^{32}	14 Tg	Mensch	35 5o Std (halbe Lebensd)		in vitro	Techn bed ngte Thrombozytenschädi gung Keine Autotransf u Oberfl Mess
1955	MORGAN u M t rb (131)	$Na_2{}^{51}CrO_4$	27 8 Tg	Kaninchen	3 4 Tg	Milz Leber Lunge (autopisch bestimmt)	in vitro	
1956	LEEKSMA u Mitarb (114 115)	DFP^{32}	14 Tg	Mensch	8 9 Tg		in vivo	Bestimmung des Abb uortes n cht mögl Nebenwirkungen ab 4mg DFP^{32}
1956	REISNER u Mitarb (168)	$Na_2{}^{51}CrO_4$	27 8 Tg	Mensch	5 8 Tg		in vitro	Initiale Sequestrat on der Thrombo zyten
1956	AAS u GARDNER (3)	$Na_2{}^{51}CrO_4$	27 8 Tg	Mensch	9 11 Tg		in vitro	Initiale Sequestrat on der Thrombo zyten
1956	ODELL (147)	S^{35} (Methionin)	87 1 Tg	Ratte	5 6 Tg		in vi o	Lange HWZ Keine Oberflächen kti tät meßb r
1957	ADELSON u Mitarb (8)	P^{32}	14 Tg	Mensch	7 Tg		in vi o	Nachte le Elution von P^{32} Keine Auto transf u Oberflächenmess ng mögl
1958	ODELL u Mitarb (148)	S^{35} (S lf t)	87 1 Tg	Mensch	5 6 Tg		in vi o	Lange HWZ H he Strahlenbelast ng Keine Oberflächenaktivität meßbar
1958	MAUPIN u Mitarb (124)	Au^{198}	2 69 Tg				in vitro	Bisher nur Mitteilung über Verwen dung als Markierungssubstanz
1961	HEYSSEL (88)	C^{14} (Seroto)	576o J	Mensch	8 14 Tg		in vivo	C^{14} Serotonin wird eluiert Ei e Ober flächenmessung ist nicht durchführb r
1963	VODOPICK u Mitarb (191)	$S35$ (Sulfat)	87 1 Tg	Mensch	8 5 Tg		in vi o	Hohe Strahlenbel stung Bestimmung des Abba ortes nicht mögl
1964	ASTER u JANDL (16)	$Na_2{}^{51}CrO_4$	27 8 Tg	Mensch	8 Tg	Leber Milz (in vivo d Oberfl Mess)	in vitro	Modifikation der Technik on AAS u GARDNER
1965	ADELSON (5)	$H^3\ DFP$	12 26 J	Hund	2 4 Tg (halbe Lebensd)		in vivo in v tro	B sher nur Ergebnis e beim Hu d publi ziert

Tab 1 Chronologische Übersicht über Methoden zur Bestimmung von Lebensdauer und Abbauort von Thrombozyten mit Isotopen

* Halbwertszeit

schen gewonnen werden konnten (11,91 92 183,185,186) Diese Untersuchungen ergaben, daß die Thrombozyten bei gesunden Personen und solchen mit einer Knochenmarksaplasie mindestens 4 bis 5 Tage uberlebten wahrend bei der idiopathischen Thrombozytopenie bereits nach wenigen Stunden alle transfundierten Thrombozyten aus dem Kreislauf verschwunden waren Entscheidender Nachteil derartiger Untersuchungen ist einmal die Notwendigkeit der Transfusion großer Plattchenmengen, die insbesondere bei der idiopatischen Thrombozytopenie mit dem Risiko einer Anaphylaxie behaftet ist (71) Bei Normalpersonen und solchen mit gering erniedrigten Werten laßt sich eine Lebensdauerbestimmung nicht exakt durchfuhren da die ubertragenen Plattchen nur bis zur Ausgangszahl verfolgt werden konnen Dasselbe gilt fur Patienten mit Polyzythamien

Alle genannten Untersuchungen ergaben keinen Aufschluß uber den Abbauort der Blutplattchen

Zu 4

Dem Franzosen JULLIARD gebuhrt das Verdienst als Erster 1950 den Versuch einer Markierung von Thrombozyten mit Radioisotopen unternommen und damit den Anstoß fur eine ganze Reihe derartiger Studien gegeben zu haben (siehe Tabelle 1) Zwar gehoren historisch diese in vitro durchgeführten Untersuchungen an den Anfang doch soll zunachst auf die in vivo –Markierungen von Thrombozyten mit Isotopen eingegangen werden

aa. Markierung von Thrombozyten im Kreislauf mit P^{32} Diisopropylfluorophosphat (DFP^{32}) und H^{32} Diisopropylfluorophosphat

P^{32} Diisopropylfluorophosphat ist ein Beta-Strahler und geht nach intravenoser Injektion eine rasche Bindung mit den Esterasen und serinhaltigen Eiweißmolekulen an den Oberflachen der Blutplattchen aber auch der Erythrozyten und Granulozyten ein (17 44) Die injizierte Radioaktivitat wird schnell ausgeschieden die Substanz als solche ist fur die Blutplattchen nicht toxisch und wird als biologisch inaktives Diisopropylphosphat metabolisiert, so daß eine erneute Markierung von Plattchen (Reutilisation) nicht stattfindet. Das Maximum der Aktivitat wird unmittelbar nach der Injektion erreicht (44) der Abfall der Aktivitatskurve ist von den meisten Untersuchern (12 114 200) als linear beschrieben worden und endet in einer terminalen Kurve so daß eine Elution des Isotops ausgeschlossen erscheint (5, 150)

Mit dieser von LEEKSMA und COHEN 1956 eingeführten Methode (114) errechnet sich die Lebensdauer menschlicher Thrombozyten auf 9-11 Tage (162 171 4a u 86b) Ihr Vorteil ist wie der aller in vivo-Methoden daß die Plattchen wahrend ihres Verbleibs im Kreislauf im Gegensatz zu den "in vitro Methoden keine Schadigung erfahren Als Nachteile dieser sonst für die Lebensdauerbestimmung geeigneten Methode sind anzusehen, daß infolge gleichzeitiger Markierung anderer Blutbestandteile die Aufarbeitung wesentlich umstandlicher ist und noch zusatzlich durch niedrige spezifische Aktivität erschwert wird (17) Weiterhin ist eine Oberflächenmessung mit Bestimmung des Destruktionsortes nicht moglich da DFP^{32} ein Beta Strahler ist Außerdem ist diese Substanz auch in Dosen von 4 mg neurotoxisch (17,77) Schließlich kann eine gleichzeitige Inkorporation in die Megakaryozyten den Untersuchungsgang storen (200)

Neuerdings haben ADELSON und Mitarbeiter an Hunden H^3 markiertes Diisopropylfluorophosphat mit gegenuber DFP^{32} 40 fach hoherer spezifischer Aktivitat verwendet und damit eine exponentiell verlaufende Lebensdauerkurve festgestellt (5 7)

Als weitere in vivo' –Technik ist die Markierung mit C^{14}–Serotonin zu erwahnen die auf der normalerweise stattfindenden Speicherung von Serotonin in den Plattchen beruht (196) Die damit beim Menschen festgestellte Lebensdauer betrug 8 bis 14 Tage (88 89) Auch mit dieser

Technik ist keine Oberflächenmessung möglich. Daneben muß die Verlässlichkeit der Methode aufgrund des nachgewiesenen Austausches des markierenden Isotops in Frage gestellt werden (157 199)

Bestimmung der Überlebenszeit durch Transfusionen von "in vivo" markierten Thrombozyten

Diese von ADELSON und Mitarbeiter 1957 (8) beim Menschen erstmals durchgeführte Technik besteht in der Verabreichung von Beta-Strahlen aussendendem P^{32}, das sich an die Phospholipoide der Blutplättchen bindet Wenn einige Tage nach der Applikation des Isotops ein Maximum an markierten Thrombozyten vorhanden ist werden diese isoliert und transfundiert Die mit dieser Methode erhaltenen Kurven zeigen die Form einer Exponentialfunktion und weisen darüberhinaus deutlich die Zeichen einer initialen Ablagerung im Gewebe (Sequestration) mit niedrigen initialen und hohen Werten nach 24 Stunden auf Beim Menschen wurden Überlebenszeiten von 5 bis 7 Tagen ermittelt (8) Da eine Elution der markierten Phosphatide gesichert ist (41 84) und die Strahlenbelastung hoch ist (0 1 mC pro kg Korpergewicht) eine Autotransfusion nicht durchgeführt werden kann und eine initiale Sequestration mit Rezirkulation eintritt kommt diese Methode für die routinemäßige Anwendung zu diagnostischen Zwecken beim Menschen nicht in Frage

ab "in vivo" Markierungen von Megakaryozyten

Fur diese Untersuchungen wurden bei Tieren bisher die Beta-Strahler $Na_2S^{35}O_4$ (146 153) C^{14} Ameisensäure und S^{35} Methionin (151,152) benutzt Nach einmaliger Injektion steigt die Radioaktivität mit zunehmender Ausschüttung markierter Thrombozyten an und fällt dann ab Die Zeit zwischen den halben Maximalpunkten des aufsteigenden und abfallenden Teiles der glockenformigen Kurve wird als Maß für die Lebensdauer angesehen Fuhrt man das Isotop dagegen taglich zu so wird das Maximum der Radioaktivität dann erreicht, wenn keine unmarkierten Plättchen mehr vorhanden sind. Dementsprechend ist die Zeit bis zu diesem Maximum die Lebensdauer Beim Menschen ist $Na_2S^{35}O_4$ bisher nur von ODELL und Mitarbeitern (148) sowie VODOPICK und Mitarbeitern (191) angewendet worden Die hohen Radioaktivitätsmengen und die lange Halbwertszeit von 87 Tagen verweisen diese Methode in den Bereich des Tierexperiments.

b "in vitro -Studien

Die Markierung von aus Blut isolierten Thrombozyten die gleichzeitig die erste Verwendung radioaktiver Isotope zur Feststellung der Lebensdauer von Blutplättchen darstellte wurde 1952 v JULLIARD und Mitarbeitern mit anorganischem P^{32} durchgeführt (27 101 102,123) und führte beim Menschen zu Zeiten von wenigen Stunden Diese verwertbaren Ergebnisse waren offenbar durch eine Thrombozytenschädigung bedingt Gleiches gilt für die ebenfalls mit P^{32} erfolgten Studien am Menschen von DESAI und Mitarbeitern (58)

An weiteren "in vitro" Untersuchungen sind die mit anorganischem P^{32} von MUELLER (133) von MORGAN und Mitarbeitern mit NaJ^{131} an Kaninchen (133) von ROBERTSON und Mitarbeitern mit $Cr^{51}Cl_3$ an Ratten (70) mit C^{14} an Ratten (153) und mit Radiogold (Au^{198}) (125) zu erwähnen.

$Na_2{}^{51}CrO_4$ wurde erstmals 1955 von MORGAN und Mitarbeitern bei Ratten benutzt (131) 1956 gaben AAS und GARDNER (2 3) sowie REISNER und Mitarbeiter (168) die heute am weitesten verbreitete und erfolgreichste Methode mit ^{51}Cr markiertem Natriumchromat an Obwohl die Verwendung dieses Isotops erstmals gleichzeitig mit der Lebensdauerbestimmung die Oberflächenaktivitätsmessung ermoglichte wurden außer vereinzelten Studien über den Abbauort der Thrombozyten (3 122) systematische

f Zu der in der Literatur in widersprechender Weise beantworteten Frage ob eine Elution von ^{51}Cr auftritt, wurde folgender Versuch durchgeführt Drei Proben mit 8×10^5 Thrombozyten wurden in 2 ml Plasma mit 20 u $C^{51}Cr$ 30 Minuten lang inkubiert, anschließend 6 mal mit physiologischer Kochsalzlösung zur Entfernung der nicht plattchengebundenen Radioaktivitat gewaschen und in 10 ml Kochsalz aufgeschwemmt. Zwei Kontrollproben wurden nach 5 Stunden, die dritte sofort nach den Aufschwemmung scharf zentrifugiert Aus jeder Probe wurden 2ml des Überstandes gemessen, wobei sich kein Unterschied in der Radioaktivitat ergab Diese Befunde entsprechen den Resultaten von ASTER und JANDL (16) und EBBE und Mitarbeitern (66) die ebenfalls keinen Abfall der Thrombozytenaktivitat in vitro beobachten konnten. Im Gegensatz dazu haben AAS und GARDNER (2) sowie DAVEY (16) bereits innerhalb der ersten Stunden eine Elution des Isotops zwischen 40 bis 70 % festgestellt, obwohl auch diese Autoren einen Austausch von ^{51}Cr in vivo nicht beobachten konnten. Auch der mit dieser Methode erzielte lineare Verlauf der Lebensdauerkurve laßt sehr vermuten daß eine Elution in vivo nicht stattfindet.

g. Daß keine Reutilisation, d.h. eine erneute Markierung von Thrombozyten durch freiwerdendes Natriumchromat beim Plattchenzerfall eintritt, ist aus folgenden Grunden anzunehmen

1 *Werden Blutplattchen aus mit ^{51}Cr* markiertem Blut am ersten Tage isoliert so enthalten sie keine Radioaktivitat
2 Selbst bei vollstandiger Zerstorung der Blutplattchen tritt kein zweiter spaterer Anstieg der Thrombozytenaktivitat ein
3 Eine Reutilisation ist auch deshalb extrem unwahrscheinlich, weil Thrombozyten in plättchenreichem Plasma weniger als 1 % Radioaktivitat binden (81)

Zusammenfassend ergibt sich aus unseren experimentellen Untersuchungen

1 Die ^{51}Cr Aufnahme in die Thrombozyten ist für den Bereich von 4^0 bis 37^0 temperaturunabhangig
2 Der Markierungseffekt nimmt mit der Dauer der Inkubation zu
3 Der Markierungseffekt steigt mit der Anzahl der Blutplattchen
4 Die absolute Menge inkorporierter Radioaktivitat steigt mit der Quantitat von Natriumchromat Der Markierungseffekt bleibt dabei unverandert
5 Der Markierungseffekt nimmt mit der *Konzentration der Blutplattchen rapide* zu
6 Eine Elution des Natriumchromat aus den markierten Thrombozyten oder eine Reutilisation findet nicht statt, so daß ^{51}Cr markiertes Natriumchromat als ein spezifischer "Tracer" anzusehen ist.

Ein Teil der Feststellungen ließ sich durch Auswertung der bei der Bestimmung der Überlebenszeit von Patienten ermittelten Thrombozytenzahlen der zugefügten Natriumchromatmengen und der Markierungseffekte überprufen Stellt man eine Beziehung zwischen Thrombozytenmenge und Chromkonzentration in der Suspension her, so zeigt sich wie in Abb 8 dargestellt eine eindeutige Korrelation zwischen plättchengebundenem ^{51}Cr / 10^{10} Thrombozyten und ^{51}Cr Menge/10^{10} Thrombozyten Diese unter *klinischen Bedingungen erhobenen Befunde* bestatigen die oben gemachte Feststellung (s.Punkt 4) einer Zunahme der Chromaufnahme in die Thrombozyten mit steigender Radioaktivitätsmenge

Aus der Abb 9 in der das plattchengebundene ^{51}Cr gegen das Produkt von Thrombozytenkonzentration und ^{51}Cr Menge aufgetragen ist ergibt sich ebenfalls eine direkte Korrelation. Ein ahnlicher Befund ist zu erheben, wenn der Markierungseffekt mit dem Produkt aus

Natriumchromatmenge und Thrombozytenkonzentration verglichen wird. (Abb 10) Der Markierungseffekt ist also um so großer je kleiner das Inkubationsvolumen ist (s. Punkt 5)

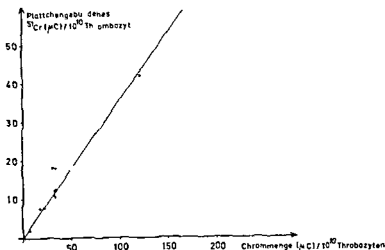


Abb 8 Plattchengebundenes ^{51}Cr im Verhältnis zur Konzentration von $Na_2{}^{51}CrO_4$

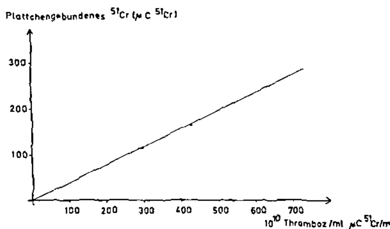


Abb 9 Plattchengebundenes ^{51}Cr in Beziehung zum Produkt aus Natriumchromatmenge und Thrombozytenkonzentration.

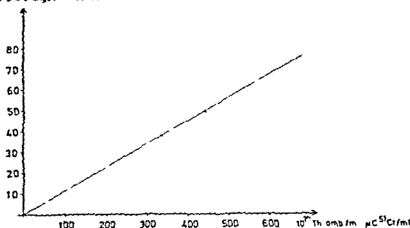


Abb 10· Markierungseffekt und Produkt aus Thrombozytenkonzentration und ^{51}Cr Menge

3 Technik der Bestimmung von Lebensdauer und Destruktionsort von Thrombozyten

Nach Phlebotomie werden 250 bis 500 ml venösen Blutes mit 25 bis 50 ml der von ASTER und JANDEL (16) beschriebenen speziellen ACD-Lösung+ in ein oder 2 evakuierte 250ml Glasflaschen gefüllt Infolge des Unterdruckes in den Flaschen ist der Blutstrom kontinuierlich und eine Gerinnung tritt nicht ein Das Verwerfen der ersten 5 ml, wie von einigen Untersuchern durchgeführt hat sich uns als unnötig erwiesen Nach guter Durchmischung des Blutes mit dem Antikoagulans wird 1 ml Blut zur pH Bestimmung entnommen, um eine eventuelle Korrektur des pH Wertes durch Zusatz von Zitronensäure vorzunehmen. In einer langsam auslaufenden Zentrifuge werden die Flaschen bei Zimmertemperatur dann 7 Minuten lang bei 500 Umdrehungen pro Minute (=56G) zentrifugiert. Das überstehende Plasma wird dann mittels eines Schlauchsystems in ein nach unten hin konisches Spitzglas überführt. Blut und Plasmamengen werden gemessen Das mit Plättchen so angereicherte erythrozytenfreie Plasma wird anschließend bei 3000 Umdrehungen/Minute ([illegible]015G) [illegible]0 Minuten lang zentrifugiert. Durch Verwendung des Spitzglases wird erreicht, daß die Thrombozyten sich zu 1 bis 2 ml am Boden komprimiert ansammeln. Das überstehende jetzt plättchenarme Plasma wird vollständig entfernt und zur späteren Verarbeitung aufbewahrt. Dem Plättchensatz werden 200 bis 300 µC ^{51}Cr in 1 ml in Form des Natriumchromates sowie 1 bis [illegible] ml plättchenarmen Plasmas zugefügt und das Gemisch gleichmäßig bis zur vollständigen Auflösung aller Plättchenklumpen aufschwemmt. Die Inkubation mit dem Isotop dauert 30 Minuten. Es erfolgt jetzt ein Zusatz von 100 ml plättchenarmen Plasmas sowie 200 mg Ascorbinsäure Durch diesen Zusatz von Ascorbinsäure wird das nicht plättchengebundene 6-wertige ^{51}Cr in 3-wertiges Chrom umgewandelt, womit eine auch durch eventuelle Spuren von freiem ^{51}Cr mögliche Markierung von Erythrozyten im Empfängerkreislauf ausgeschlossen wird (167) Je 2 x 1 ml dieser Aufschwemmung (Probe A) werden entnommen. Die Plättchenaufschwemmung wird nun erneut bei 3 000 Umdrehung / Minute 30 Minuten lang zentrifugiert Der überstehende jetzt plättchenarmen Flüssigkeit wer

+ Je 2x1 ml [illegible]
0 065 mol. Dextrose [illegible]

den je 2 x 1 ml zur Bestimmung der nicht an die Thrombozyten gebundenen Radioaktivität entnommen (Probe B) im übrigen wird sie vollständig entfernt Man schwemmt jetzt den Satz markierter Thrombozyten in etwa 20 ml des aufbewahrten plättchenarmen Plasmas unter Beachtung einer vollständigen Agglutinationsfreiheit auf Aus dieser endgültigen Suspension werden zur Bestimmung der insgesamt zur Injektion gelangenden Radioaktivität je 0 5ml in 1 Röhrchen mit 3ml physiologischer Kochsalzlösung (Probe C) u in 1 Röhrchen mit 3ml 1%iger Natriumoxalatlösung (Probe D) gegeben Beide Proben werden 30 Minuten lang bei 3000 Umdrehungen/Minute zentrifugiert, der Überstand vollständig entfernt Da Natriumoxalat die Erythrozyten sofort auflöst läßt sich aus dem mit Natriumoxalat bearbeiteten Thrombozytensatz leicht die an die Thrombozyten gebundene Aktivität errechnen Demgegenüber ergibt sich aus dem anderen Röhrchen die gesamte injizierte Radioaktivität Unmittelbar nach Fertigstellung der endgültigen Thrombozytensuspension wird diese injiziert Die Markierung von Blutplättchen ist somit wesentlich aufwendiger als die von Erythrozyten und dauert 2 - 3 Stunden Vier Stunden sollten wegen der mit zunehmen der Dauer der Verarbeitung eintretenden Thrombozytenschädigungen nicht überschritten werden

Die Messung der Blut Plasma- und Thrombozytenproben wurden mit dem Tracer lab SC 57 A Low Background Well Scintillation Counter druchgeführt Die Leerwerte betrugen zwischen 50 bis 90 Impulse/Minute Aus mehreren Meßwerten wurden die Mittelwerte für die Berechnungen herangezogen Zur Bestimmung der Thrombozytenzahlen in den entsprechenden Flüssigkeiten wurde die Methode von BRECHER und CRONKITE im Dunkelfeld benutzt (36)

4 Bemerkungen zur Technik

Gefäße

Wegen der bei Berührung mit Glas angeblich eintretenden Thrombozytenschädigung (140) haben die meisten Untersucher (16,57 143) bisher silikonisierte Gefäße oder Plastikbehälter benutzt Aus den Untersuchungen von McILVANIE (127) insbesondere aber denen von KISSMEYER -NIELSEN (104 105) und anderen (46, 107) geht jedoch einwandfrei hervor daß Glas, silikonisierte Behälter oder Plastikbeutel keinen unterschiedlichen Einfluß auf die Lebensdauer der Thrombozyten haben. Das wird durch die eigenen Befunde bestätigt Vielmehr soll die Retraktion und die C^{14}-Serotonin-Aufnahme von Blutplättchen die in Plastikbehältern aufbewahrt wurden stärker gestört sein (156)

Thrombozytenpräparation

Frühere eigene Versuche haben gezeigt daß bei Zentrifugation der von uns benutzten 250ml Flaschen die größte Ausbeute (=Extraktion) bei einer Umdrehungszahl von 500/Minute nach 4 bis 5 Min erzielt wird. Zu dieser Zeit ist die Erythrozytensedimentation noch nicht vollständig erfolgt. Der nach 7 Minuten erreichte Extraktionseffekt (das Verhältnis der Blutplättchenzahl im plättchenreichen Plasma zu der im Vollblut) betrug bei 37 Personen durchschnittlich 67 7% (31 1 bis 98 3%) Es wurden durchschnittlich 8 27 x 10^{10} Thrombozyten für die Markierung benutzt

Ohne Zweifel besteht eine der Hauptschwierigkeiten der Markierung von Blutplättchen in ihrer Verletzlichkeit. Obwohl eigene Untersuchungen zur Bedeutung des Antikoagulans und des pH nicht durchgeführt wurden muß hier kurz darauf eingegangen werden Übereinstimmend ist bei allen Untersuchungen von mit Aethylendiamintetraessigsäure (EDTA) als Antikoagulans aufgeschwemmten Thrombozyten

festgestellt worden daß die Blutplättchen innerhalb von 10 Minuten bis zu 95% aus der Blutbahn verschwinden (16 20 108) unter Erreichung ihres Maximums von etwa *30% (60 75)* nach 4 bis 6 Stunden bis 24 Stunden im Kreislauf wieder erscheinen und dann normal uberleben Dieser initiale Tiefpunkt ist Ausdruck einer partiellen Ablagerung die vorwiegend in der Leber stattfindet (16) Daß diese Sequestration und der gleichzeitig zu beobachtende geringe Prozentsatz lebensfähiger Thrombozyten Folge ihrer Schädigung durch EDTA sind (16,104), laßt sich einmal durch die morphologisch sichtbare Umwandlung der normalerweise vorhandenen Scheibenform in die Kugelform, zum anderen durch die Irreversibilitat nach Waschen mit der eine Thrombozytenschadigung weitgehend vermeidenden „sauren" ACD-Losung belegen. Da niedriger Plattchenertrag und Sequestration aber auch bei Verwendung normaler ACD-Losung beobachtet wird (42), und durch Verminderung des pH-Wertes auf 6 3 bis 6,5 infolge entsprechender Veranderung der ACD-Losung ein hoherer Plattchenertrag erreicht und die initiale Sequestration vermieden werden kann (16 42) scheint die Senkung des pH Wertes der entscheidende die Thrombozytenschadigung vermeidende *Faktor zu sein Dafur* spricht auch, daß bei einem pH Wert von 6 5 die Adhasivitat der Blutplattchen an Oberflachen die eine Resuspension unmoglich machen wahrscheinlich infolge des dabei geringeren Zerfalls von ATP zu dem die viskose Metamorphose einleiten den ADP herabgesetzt wird (166)

Bei unseren Untersuchungen lag der pH Wert immer zwischen 6 2 und 6,5 Auf die damit erzielten Plattchenertrage wird spater eingegangen Die endgultigen Suspensionen wurden auf Agglutinate untersucht. *Sie ließen sich nicht beobachten* Die Thrombozyten hatten Scheibenform

Aufschwemmungsmedium

Im Gegensatz zu AAS und GARDNER (2) hat DAVEY (16) bei Inkubation mit NaCl bessere Markierungseffekte erzielt Die von ihm berichtete Bindung des Natriumchromat an Plasmaproteine fallt u E nicht ins Gewicht da die von uns erzielten *Markierungseffekte* bei Inkubation mit Plasma außerordentlich hoch sind s Seite 23 Das Inkubationsvolumen betrug 2 bis 5 ml

Mehrfaches Waschen der Thrombozyten schadigt diese und fuhrt leicht zu Verklumpungen. Daß durch Zusatz von 100ml plattchenarmen Plasmas nach der Inkubation die freie Radioaktivitat weitgehend beseitigt wird, laßt sich durch die niedrigen Plasmaradioaktivitatswerte nach Injektion (Tab 6) und durch die geringen Radioaktivitatsausscheidungen im *Urin* bei unseren Patienten (s Tab 5) belegen

5 **Berechnungen der Untersuchungsergebnisse**

a Berechnung des Markierungseffektes
Als Markierungseffekt wird das Verhaltnis an die Thrombozyten gebundene ^{51}Cr Menge zu der insgesamt inkubierten Radioaktivitatsmenge bezeichnet Es wird in Prozent ausgedruckt und laßt sich auf verschiedene Weise berechnen

$$\text{I} \quad 100 \quad \frac{\text{Imp/min (Probe B)} \times 100}{\text{Imp/min (Probe A)}}$$

$$\text{II} \quad \frac{2 \times \text{Imp/min (Probe C)} \times \text{ml d.inj.} \text{Thromb aufschwemm} \times 100}{\text{Imp/min (Probe A)} \times 100}$$

$$\text{III} \quad \frac{2 \times \text{Imp/min (Probe D)} \times \text{ml. d inj.} \text{Thromb aufschwemm} \times 100}{\text{Imp/min (Probe A)} \times 100}$$

Bei der Berechnung des Markierungseffektes auf die 3 angegebenen Arten, die eine gegenseitige Kontrolle darstellen hatten die Ergebnisse zwischen I II und III durchschnittlich eine Differenz von 8? Bei 41 Untersuchungen

betrug der Markierungseffekt zwischen 4,3 bis 64 5% im Durchschnitt 37,3% Ein Markierungseffekt unter 10% wurde nur einmal unter 20% insgesamt 3 mal erzielt

b Die injizierte Radioaktivitatsmenge

Das Produkt aus der Impulsrate/ml der mit Natriumoxalat und mit physiologischer NaCl aufgearbeiteten Proben mit der Menge der endgultigen Aufschwemmung bildet die gesamte an die Thrombozyten gebundene Radioaktivitat und die insgesamte injizierte Aktivitat Die Messung dieser beiden Grossen war erforderlich zur Feststellung, ob die Beimischung von minimalen noch verbliebenen Erythrozytenmengen nach Präparation der Thrombozyten (im Mittel 5 x 10^3 bis 5 x 10^5 Erythrozyten) für die Bedeutung der Lebensdauer und insbesondere des Destruktionsortes hatten Es ergab sich dabei kein wesentlicher Unterschied Die injizierte Radioaktivitätsmenge betrug durchschnittlich 124 μ C ^{51}Cr

c Die Berechnung der Thrombozytenlebensdauer

5 - 10 Minuten 30 Minuten 2 Stunden und 1 oder 2 taglich nach Injektion der endgültigen Thrombozytensuspensionen werden 12 ml Blut gerinnungsfrei abgenommen Aus 2 ml Blut kann die Blutradioaktivität bestimmt werden Aus den restlichen 10 ml wird durch Differenzialzentrifugation plättchenreiches Plasma gewonnen Der nach 30-minutiger Zentrifugation bei 3000 Umdrehungen/Minute erhaltene Plättchensatz wird von dem überstehenden Plasma getrennt Bei einem Teil der Patienten haben wir dieses plattchenarme Plasma nochmals 1 Stunde bei 18000 Umdrehungen/Minute zentrifugiert und die freie Plasmaaktivität bestimmt (s. Tab 6) Fur die Thrombozytenlebensdauerkurve wird der initiale Aktivitätswert als 100 %-Wert zugrunde gelegt und die Werte der anderen Proben rechnerisch in % zum Ausgangswert angegeben. Die auf diese Weise ermittelte Kurve setzt sich zusammen aus dem spontanen Aktivitätsabfall des Isotops und dem Zerfall der Blutplättchen Der spontane Aktivitätsabfall muß dementsprechend berücksichtigt werden Die Ausgangsaktivität (n_0) fällt zum Tage T (n_t) entsprechend der Exponentialfunktion

$$n_t = n_0 x e^{-a x t} \quad (1)$$

ab wobei a x t die Zerfallskonstante am Tage T ist.

Bei Berücksichtigung der Halbwertszeit von ^{51}Cr von 27 8 Tagen gelangt man zu einer Zerfallskonstante von 0,025 Nachdem - wie oben dargestellt - unsere absolut gemessenen Ausgangsaktivitäten grundsatzlich mit 1000 Impulsen/Min gleichgesetzt wurden, gestaltet sich die Gleichung (1) folgendermaßen

$$n_t = 1000 x e^{-a x t} \quad (2)$$

Der sich aus dem Spontanzerfall errechnende Quotient von Ausgangsaktivität zu Restaktivität stellt denjenigen Faktor (F_t) dar mit dessen Hilfe man den nur von der Thrombozytenlebensdauer abhängigen Radioaktivitätsverlust nach mathematischer Korrektur für den jeweiligen Spontanaktivitätsverlust des Isotops nach folgender Gleichung berechnen kann

$$A_t = a_t \times F_t \quad (3)$$

Fur die tägliche Änderung des Faktors F_t gibt es bekannte Tabellen (51) Als Thrombozytenlebensdauerkurve wurde die Zeit angegeben, nach der die nach Gleichung (3) berechnete Aktivitat den 10 %-Wert der Ausgangsaktivität erreicht hatte Der spontane Radioaktivitätsabfall kann dann unberücksichtigt bleiben, wenn alle Proben am selben Tag gemessen werden.

d Messung der Oberflächenaktivität

Entsprechend den Zeiten der Blutabnahme wurden die Oberflachenaktivitätswerte über Herz, Leber, Milz und Knochenmark gemessen Wahrend wir zunächst die erste Messung nach 30 Minuten durchführten, haben unten noch näher auszuführende Beobachtungen uns veranlaßt, die Messungen während der Injektion der markierten Blutplättchen vorzunehmen. Die unmittelbar nach Injektion durchgeführte Messung ist deshalb von besonderer Bedeutung, weil sie interessante Rückschlusse auf das Verhalten der Thrombozyten in der ersten halben Stunde erlaubt. Als Meßgerat wurden der Szintillationszahler der Fa FRIESECKE und HÖPFNER G m b H mit einem Natrium-Jodid Kristall von 3,0 x 2,0 cm und das Strahlungsmeßgerat FH 49 verwandt. Als Bleiabschirmung diente das Siemens Nukleoskop

Folgende Meßpunkte wurden für den tangential aufgesetzten Meßkopf gewahlt

Herz	4 I C R. links parasternal
Leber	Schnittpunkt der rechten Medioclavicularlinie mit der untersten Rippe
Milz	Schnittpunkt der mittleren linken Axillarlinie mit der 10 Rippe in Rechtsseitenlage
Knochenmark	Os sacrum in Bauchlage

Entscheidend für die Reproduzierbarkeit und die Auswertung der Ergebnisse ist, daß der Szintillationszahler immer an derselben Stelle aufgesetzt wird Dabei bleiben die für die tatsachlich gemessenen Aktivitatswerte bedeutenden Faktoren konstant namlich die Absorption durch das durchstahlte Gewebe die Streustrahlung und der Abstand des Szintillationszahlers vom gemessenen Organ Bei Vorliegen von Organvergroßerungen wurde an mehreren definierten Stellen des Organs gemessen Zur Auswertung der gemessenen Oberflachenaktivitat wurde das von HUGHES-JONES und SZUR zur Erythrozytenmarkierung angegebene Verfahren (95) angewandt Dabei wurden die tatsächlich gemessenen hochsten Aktivitatswerte unter Berucksichtigung des Leerwertes über dem Herzen in jedem Falle auf 1000 Impulse/Minute umgerechnet, um so verschiedene Personen vergleichen zu konnen Die ubrigen gemessenen Organaktivitaten wurden unter Einbeziehung des spontanen Aktivitätsverlustes entsprechend den Verhaltnissen bei der Thrombozytenlebensdauerkurve (siehe Gleichung 3) auf diesen Herzwert bezogen und entsprechend umgerechnet Die Bedeutung der Organgroße und der Durchblutung, die sich in den absoluten Impulszahlen widerspiegeln und so eine bedeutende Rolle bei ausschließlicher Beachtung der tatsachlich gemessenen Aktivitats-Werte spielen konnen, lassen sich durch die Bestimmung der zusätzlichen Aktivitatswerte ausschließen

Damit ist der gegenuber dem Aktivitatsverlauf uber einem nicht thrombolytisch aktiven Organ tatsachlich eingetretene Aktivitatsanstieg oder abfall uber Leber, Milz und Knochenmark gemeint Als Vergleichsorgan wird das Herz gewahlt

Der theoretisch uber einem Organ zu erwartende Aktivitätswert (W) am Tage t betragt

$$W = W_o - W_o \times \frac{H_o - H_t}{H_o} \qquad (5)$$

wobei W_o der Ausgangswert uber dem Organ H_o der Ausgangswert über dem Herzen, H_t der Aktivitatswert am Tage t uber dem Herzen ist Die zusätzlichen Aktivitatswerte (ZA) errechnen sich dementsprechend nach der Formel

$$ZA = W_o \times \frac{H_o \; H_t}{H_t} + W_t \;\; W_o \qquad (6)$$

W_t ist der am Tage t tatsächlich gemessene Oberflächenaktivitätswert. Auf die Bedeutung der zus Aktivitätswerte für die Beurteilung von Abbau u /oder Sequestration wird später eingegangen

e Berechnung des Plättchenertrages

Unter Plättchenertrag - im angloamerikanischen Schrifttum als "recovery" bezeichnet - versteht man diejenige thrombozytär gebundene Radioaktivitätsmenge, die nach Injektion der markierten Thrombozyten noch im Kreislauf vorhanden ist, ausdrückt in Prozent der injizierten ^{51}Cr Radioaktivität Er berechnet sich nach folgender Formel Radioaktivität des Plättchenertrages von 1 ml Blut mal Plättchenextraktion mal theoretisches Blutvolumen durch gesamte injizierte Radioaktivität
Als theoretisches Blutvolumen wurde 70 ml/kg Körpergewicht zugrunde gelegt (82) Die Plättchenextraktion wurde bei jeder Probe gemessen

f Strahlenbelastung

Unter der Annahme einer Gesamtkörperdosis von 0 2 rad pro $mC^{51}Cr$ (55) und Zugrundelegung der von uns injizierten Radioaktivität von 124 μ $C^{51}Cr$ lag die Strahlenbelastung bei einem normalen Erwachsenen bei maximal 25 mrad Bei dieser Dosis handelt es sich nach den Bestimmungen der internationalen Strahlenschutzkommission 1958 um 1/10 der für Ganzkörperbestrahlung maximal erlaubten Dosis MOLLISON und VEALL (130) haben für mit Natriumchromat markierte Erythrozyten berechnet daß selbst bei vollständiger Zerstörung aller Blutkörperchen am ersten Tage eine Dosis von 300 μ C ^{51}Cr unter Berücksichtigung von Organverteilung und Strahlungsart sowie Halbwertszeit erlaubt ist Bei allen Untersuchungen wird die Dosis aber nicht nur durch die maximale Gesamtkörperdosis sondern diejenige des kritischen Organs limitiert Diese Menge beträgt für das bei Studien mit ^{51}Cr kritische Organ Niere nach RAJEWSKI 600 μ C ^{51}Cr (165) sodaß wir also im Durchschnitt mit 1/5 der maximal zu lässigen Dosis gearbeitet haben Selbst bei Beachtung der bei einer chronischen idiopathischen Thrombozytopenie besonders starken Destruktion der Thrombozyten in Milz und Leber liegt diese Menge weit unterhalb der für diese Organe zulässigen Dosen

Natriumchromat ist eine für Zellbestandteile keineswegs neutrale Substanz und führt so bei Inkubation mit Erythrozyten jenseits einer Konzentration von 22 Gamma pro ml zur toxischen Schädigung infolge Methämoglobinbildung (98) Obwohl Untersuchungen hierzu nicht vorliegen, waren dementsprechend auch für Thrombozyten unter Umständen Schädigungen zu erwarten zumal die Inkubation im Satz durchgeführt wurde und so eine relativ hohe Natriumchromatkonzentration pro Plättchenzahl vorhanden war Aus den von uns erzielten Thrombozyten lebensdauerkurven und Plättchenertragswerten sowie der in Abbildung 11 dargestellten regellosen Verteilung der Markierungseffekte in Beziehung zur absoluten im Natriumchromat enthaltenen Chromkonzentration in Gamma pro 10^{10} Thrombozyten geht hervor, daß bei Chrommengen von insgesamt 0 47 bis 6 06 Gamma bzw Chromkonzentrationen bis zu 1 Gamma pro 10^{10} Thrombozyten in maximal 5 ml Inkubationsflüssigkeit sicher keine die Lebensfähigkeit beeinträchtigende Wirkungen durch Natriumchromat auftreten Die in Abbildung 11 gezeigte Verteilung hängt wahrscheinlich mit der unterschiedlichen spezifischen Aktivität von 78 mC/mg Cr bis 347 mC/mg Cr zusammen.

Möglicherweise steht die von anderen Autoren diskutierte Toxizität von ^{51}Cr im Zusammenhang mit den relativ niedrigen spezifischen Aktivitäten (20 bis 30 mC/mg Cr) wodurch naturgemäß

hohere Chromdosen zur Anwendung kommen

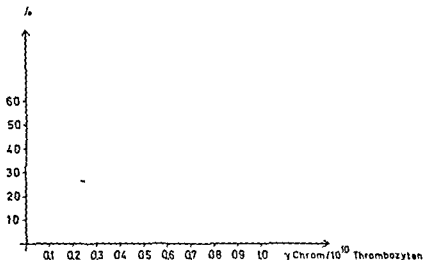


Abb 11 Markierungseffekt bei verschiedenen Konzentrationen von Natriumchromat.

A Kontrollpersonen

Bei 19 hämatologisch gesunden Personen wurden die Überlebenszeit und die Oberflächenaktivität bestimmt. Die dabei ermittelten Werte sind in Tabelle 3 zusammengestellt.
Die normale Thrombozytenlebensdauer kurve nach autologer Transfusion ist in Abbildung 12 dargestellt Das Maximum der im Vergleich auf 1000 Impulse /Minute umgerechneten Radioaktivität liegt zu Beginn Die Kurven fallen dann praktisch linear bis zu einem Wert von 10% der Ausgangsaktivität ab um von diesem Punkt aus in einen exponentiellen Teil überzugehen der nach maximal 14 Tagen die Nullinie erreicht. Praktisch alle Untersucher die mit der sauren ACD-Lösung von ASTER und JANDL gearbeitet haben (16,31,32,33, 78) haben diesen Kurvenverlauf beschrieben In Übereinstimmung mit DAVEY und NAJEAN (55 143) haben wir einen Unterschied weder hinsichtlich der Kurvenform noch der Dauer der Thrombozytenlebenszeit zwischen autologer und homologer Transfusion (Fälle 6 9 und 12) gesehen Sowohl Kurvenform als auch Dauer sind reproduzierbar und vom Absolutwert unabhängig vergleichbar

Bei Berechnung auf 100 % als Ausgangswert verläuft die gemessene Blut aktivität der Kurve der Thrombozytenlebensdauer parallel
(Abbildung 13)

Hinsichtlich der Länge der Lebensdauer sind von den verschiedenen Au oren Zeiten zwischen 7 und 14 Tagen angegeben worden je nachdem ob die Kurve vom linearen Teil aus extrapoliert oder der vollständige Aktivitätsschwund bemessen wurde Da der oben beschriebene Verlauf grundsätzlich - mit Ausnahme extremer Verkürzungen - auch bei verringerten Thrombozytenlebensdauer beobachtet wird (s. Abbildung 24) und die nach dem Knick der Kurve verbleibende Restaktivität unterschiedlich flach verläuft so daß Abweichungen von 2 - 3 Tagen resultieren, haben wir als Maßstab der Thrombozytenlebensdauer den 10%-Wert der Ausgangsaktivität angesetzt (32) Neuerdings hat BALDINI (18) ebenfalls eine derartige Beurteilung vorgeschlagen Bei Zugrundelegung dieser Bewertung haben wir bei hämatologisch gesunden Kontrollpersonen die Thrombozytenlebensdauer mit 8,3 (7,2 9 9) Tagen in Übereinstimmung mit AAS und GARDNER (65) ASTER und JANDL sowie DAVEY und LANDER und anderen (16,39,57) ermittelt Aus dem Verlauf der Kurve ist weiterhin ein täglicher Abfall von rund 12% zu entnehmen.

Der exponentielle Formverlauf im letzten Abschnitt der Überlebenszeit kurven hat zu Diskussionen Anlass gegeben Neben Verbleib langer lebender jugendlicher Thrombozyten (8) ist an eine Reutilisation des Isotops gedacht worden Andererseits bestehen Hinweise daß es sich hier um eine andere Thrombozytenpopulation handeln konnte (195) Eine ausreichende Erklärung dieses von allen Untersuchern festgestellten Phänomens ist bisher nicht vorhanden

Während der ersten Stunden ist der Abfall der Radioaktivität in der Regel relativ gering. Ganz selten sieht man einmal bei Verwendung des besagten Antikoagulans einen initialen Anstieg, der aber praktisch nie 10% übersteigt Übereinstimmend haben alle Autoren die mit EDTA oder normaler ACD-Lösung gearbeitet haben einen Anstieg

Nr	Name	Alter J	Geschl	Diagnose	Art der Markierung Autolog =A Homolog =H	Thrombozyten/mm^3	Markierungseffekt	Plättchenertrag (%)	Plättchenertragsfl (cm^2)	Thrombozytenlebensd (Tg)
1	K W	48	m	Zstd n Pleuropneumonie	A	250 000	-	50 0	109 94	9 9
2	B E	43	m	Zstd n Enteritis	A	198 000	-	48 0	93 51	9 0
3	A A	32	m	Zstd n Enteritis	A	213 000		56 0	102 63	7 7
4	K St	39	m	Mitralstenose	A	184 000	-	42 0	67 26	8 3
5	Sch E	22	m	Akzident systol Geräusch	A	184 000	-	44 4	90 96	9 7
6	T W	31	m	Komb Mitralvitium	H	193 000	-	56 0	87 44	7 7
7	P I	64	m	Gastritis	A	174 000	-	42 0	81 18	7 0
8	M P	41	m	Magenulcus	H	189 300	23 3	38 3	89 90	8 6
9	E I	65	m	Zstd n Bronchpneumonie	A	346 000	55 4	44 1	81 72	7 2
10	B E	36	m	Vegetative Dystonie	A	224 000	19 0	40 0	90 60	8 4
11	L D	25	m	Schlafmittelintoxikation	A	255 000	-	65 0	119 08	7 5
12	G W	70	m	Duodenaldivertikel	H	198 000	-	59 0	126 61	8 6
13	R H	59	m	Vegetative Dystonie	A	283 000	26 2	70 2	152 42	7 8
14	S W	51	m	Vegetative Dystonie	A	358 000	25 9	71 0	124 87	7 6
15	A B	64	m	Hypertonie	H	165 000	-	45 0	97 60	9 2
16	H A	36	m	Holzbeizenvergiftung	A	221 000	-	53 0	116 22	8 5
17	D R	34	m	Leichte Mitralinsuffizienz	A	233 000	-	57 5	148 33	8 7
18	R K	21	m	rheum Polyarthritis	A	273 000	44 0	41 0	75 13	8 4
19 *	v d W W	24	m	Osteochondrose	A	258 000	18 6	36 0	62 69	5 8
20 *	G R	37	m	Zstd n Co Vergiftung	A	223 000	30 8	46 6	73 42	6 5
21	F W	54	m	Nierenbeckenstein	A	224 000	48 4	94 0	226 43	9 2

Bei den mit * gekennzeichneten Personen wurden Untersuchungen mit Übertragung von ITP Plasma vorgenommen

Tab 3 Thrombozytenlebensdauer bei Kontrollpersonen.

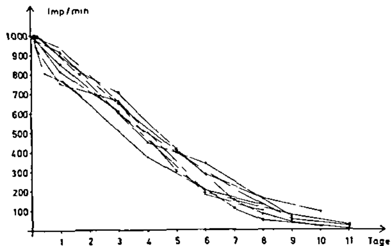


Abb 12 Thrombozytenlebensdauer bei 8 Kontrollpersonen.

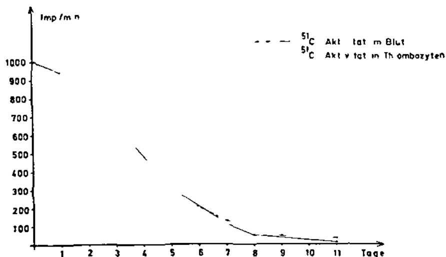


Abb 13 Blut und Thrombozytenradioaktivitatskurve bei einer Kontrollperson.

der Thrombozytenlebensdauerkurve von tiefen auf höchste Werte nach 24 und bisweilen 48 Stunden gesehen So hat DAVEY (55) den Verlauf der Thrombozytenlebensdauer in 4 Stadien eingeteilt

1 Auftreten der Thrombozyten im Kreislauf
  0 bis 5 Minuten nach Injektion
2 Verschwinden der Blutplättchen nach 5 bis 30 Minuten
3 Rezirkulation nach 30 Minuten bis 24 Stunden und
4 Überlebenszeit

In Kenntnis der Eigenschaften "saurer' ACD-Lösung haben wir derartige Untersuchungen nicht angestellt Denn es kann aufgrund der Untersuchungen von ASTER und JANDL (16) sowie COHEN und Mitarbeitern(42) heute keinem Zweifel unterliegen daß es sich bei diesem von DAVEY ausführlich beschriebenen Phänomen um Artefakte infolge einer Thrombozytenschädigung durch ungenügende Senkung des pH und möglicherweise nicht ausreichen de Zitratkonzentration handelt Für praktische Zwecke ist daraus zu fol gern daß so behandelte Thrombozy tenaufbereitungen hämostyptisch zu nächst nicht und insgesamt vermindert zur Auswirkung gelangen Dement sprechend sind auch die mit so präpa rierten Blutplättchenmarkierungen er zielten Oberflächenaktivitätsmessungen hinsichtlich des besonders bei verkürz ter Lebensdauer gerade in den ersten Stunden wesentlichen Verhaltens nur mit den größten Vorbehalten zu betrachten

Zur Annahme die mittels ^{51}Cr Mar kierung erhaltenen Thrombozytenle bensdauerkurven als echt zu betrach ten, ist man aus folgenden Gründen berechtigt

Die Thrombozytenzahlen von den eingangs erwähnten mit hohen unter Um ständen letalen Strahlendosen bela steten Personen nehmen nicht nur grundsätzlich den gleichen Verlauf sondern sie erreichen auch nach einer Zeit von etwa 7 - 10 Tagen Minimalwerte (87 120) Zum anderen stimmen die mit dieser Methode erzielten Werte mit dem nach Markierung in vivo mit DFP 32 bei dem bekanntlich weder eine Thrombozytenschädigung noch eine Elution des "Tracers" eintritt, mit unseren Befunden überein (114)
Schließlich werden bei erstmaligen Transfusionen von Plättchen auf extrem thrombozytopenische Patienten mit Markaplasie gleiche Resultate er zielt (20) In Übereinstimmung mit durch ^{51}Cr und DFP32-Markierungen erhobenen Befunden anderer Autoren (16,20,143,144,145,200) haben wir einen praktisch in Abhängigkeit von der Zeit linearen Abfall der Aktivität festgestellt Die Kurven zeigen mathematisch in der Gesamtzahl den Verlauf "O ter Ordnung' Bei Annahme eines Thrombozytenverbrauches wäre dem gegenüber das Auftreten einer Abfallkurve 1 Ordnung zu erwarten, die also exponentiell verliefe Für die so mit P^{32}(8) und C^{14} Serotonin Markier ungen (88,157) aufgezeichneten exponentiell verlaufenden Kurven läßt sich sagen, daß sie auf einer Elution von P^{32} (69,84) und C^{14}-Serotonin (157) beruhen und dementsprechend nicht den wahren Kurvenablauf darstellen Gegen den Verlauf aufgrund einfacher Alterung sind besonders tierexperimen telle Befunde von ADELSON und Mit arbeitern (6,9) erhoben worden, die bei der Beobachtung von gleichaltrigen Thrombozyten-"Kohorten eine Ver längerung der Überlebenszeit unter Heparin- und Dicoumaroltherapie fest gestellt haben MURPHY und MUSTARD haben anhand größerer stati stischer Erhebungen bei Patienten mit Arteriosklerose eine verminderte, bei solchen unter Antikoagulantientherapie eine verlängerte Thrombozyten lebensdauer beobachtet und daraus den Schluß gezogen daß die Überlebenszeit kurve Ausdruck eines hämostatischen Verbrauchsprozesses sei (134 135 136 137 138 139) Abb 14 zeigt die Kur en

von 5 Patienten die wegen eines Herzinfarktes unter Marcumarbehandlung im therapeutischen Bereich eines Prothrombinspiegels von 15 - 25% standen Die durchschnittliche Lebensdauer von 8,3 Tagen weist keinen Unterschied gegenuber den Kontrollbefunden auf Das entspricht den Befunden anderer Autoren, die keinen Unterschied zwischen der Lebenszeit normaler Personen und Hamophiler sowie Arteriosklerotiker (21,55 154) und solcher mit Thrombasthenie(143) gesehen haben Außer den erwahnten sich widersprechenden Befunden bei Hypo- und Hyperkoagulabilitat des Blutes liegen mit Ausnahme der selbstverstandlich bei schweren unter Umständen generalisierten Thrombosen im Einklang mit einer klinisch zu beobachtenden Thrombozytopenie verkurzten Thrombozytenlebensdauer (38) keine Beweise dafür vor, daß sich der Abbau bei Normalpersonen infolge Verbrauch vollzieht. Daß der Verlauf der Thrombozytenlebensdauerkurve annahernd linear ist und auch bei Markaplasie mit Thrombozytopenie die Überlebenszeit normal gefunden wird, obwohl die dabei für die verschiedenen Gerinnungsprozesse im Korper insgesamt zur Verfügung stehende Thrombozytenmenge vermindert ist, sind entscheidende Gründe die annehmen lassen daß der Abbau der Thrombozyten normalerweise entsprechend ihrem Alter vor sich geht

Plattchenertrag

Der Plattchenertrag d h der Prozentsatz im Kreislauf befindlicher markierter Plattchen an der gesamten injizierten Radioaktivität drückt gegenuber der Thrombozytenlebensdauerkurve, die ja nur Relativwerte angibt die wahren Verhältnisse aus Grundsätzlich muß die Aufzeichnung der Plättchenertragswerte einen der Überlebenszeit identischen Verlauf nehmen da sie sich aus den markierten Thrombozyten berechnen Abb 15 zeigt eine Kurvenschar bei Normalpersonen Der Plattchenertrag betrug bei hamatologisch Gesunden durchschnittlich 56 1% (36 - 94%)

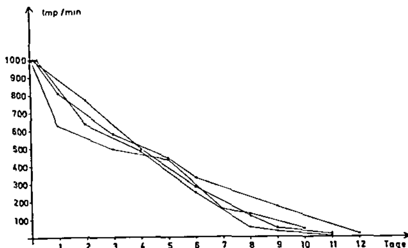


Abb 14 Thrombozytenlebensdauer bei 4 Patienten wahrend Antikoagulantientherapie.

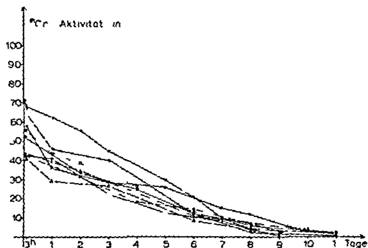


Abb 15 Plättchenertrag bei 9 Kontrollpersonen.

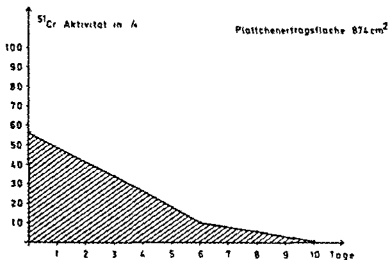


Abb 16 Schematische Darstellung der Plättchenertragsfläche bei einem Normalfall.

Aus dem ermittelten Plattchenertragswert leitet sich her daß etwa 40-45% der markierten *Blutplättchen im Kreis* lauf nicht zirkulieren und zwar - wie der sich dann gleichmaßig vollziehende Abfall der Kurve beweist nicht infolge zeitlich begrenzter Ablagerung im Gewebe sondern infolge irreversibler Schädigung Der Plattchenertrag ist damit zugleich eine wichtige Große zur Beurteilung der Lebensfähigkeit der Thrombozyten

Da Dauer und Form der Überlebenszeit sowie Plättchenertrag Faktoren darstellen die sich unabhängig voneinander bei verschiedenen Erkrankungen verändern konnen war es erstrebenswert, sie zu einem einheitlichen Parameter zusammenzufassen Bei dieser von BALDINI (20) eingeführten Große handelt es sich um die Fläche die sich unterhalb der Plättchenertragswertkurve zwischen Ordinate und Abszisse befindet (Abb 16) Bei Anwendung eines definierten Koordinatensystems (2 5 cm Abszisse/Tag und 2 cm Ordinate/10% Plattchenertrag) wurde bei Normalpersonen ein Flachenwert von 60 cm^2 nie unterschritten Diese Art der Vermessung gestattet - wie später ausgeführt wird - auch bei normalen initialen Ausbeutewerten und verkürzter Lebensdauer eine bedeutsame Aussage

Abbauort

Wie die Oberflachenaktivitatswerte bei Normalpersonen sich verhalten ist in Abb 17 aufgezeichnet Man sieht daß die Herzaktivitat langsam abfällt Der Verlauf der Aktivitatskurve über dem Herzen geht der mit dem Blut wider Erwarten nicht parallel. Das hangt wahrscheinlich mit einer partiellen Benetzung der Wände des Herzens mit markierten Thrombozyten zusammen Die Aktivitat uber der Leber fällt ab und steigt dann insgesamt langsam gering an oder bleibt konstant Über der Milz nehmen die Aktivitatswerte dagegen bereits in den ersten Minuten als Ausdruck einer starken Radioaktivitatsansammlung schnell zu und steigen in den nächsten Tagen dann ebenfalls gering an oder bleiben konstant Die Knochenmarksoberflachenaktivität ist wesentlich geringer und fallt im ubrigen in einigen Tagen langsam ab Messungen in den ersten Minuten (Abb 18) lassen erkennen daß der initiale Anstieg über der Milz bei Normalpersonen praktisch immer nach 12 bis 17 Minuten abgeschlossen ist Er ist Ausdruck der Ablagerung der bei der Markierung geschädigten Thrombozyten Da zu dieser Zeit bereits nur noch 55% der markierten Thrombozyten lebensfähig im Kreislauf zirkulieren ist anzunehmen daß vor allem die Milz u. zu einem geringeren Teil die Leber Grabstatte der restlichen 45% sind Wie aus den Abb 17 und 18 zu entnehmen ist kommt es danach nicht zu einem Wiederabfall der Aktivitat über der Leber oder der Milz wodurch sich dieser Aktivitätsverlauf grundsatzlich von der bei EDTA reversiblen Sequestration in Leber und Milz unterscheidet Der initiale Aktivitätsanstieg uber der Milz ist vielmehr eine bei der Markierung mit ^{51}Cr in unserer Technik regelhafte Erscheinung, die von den durch Destruktion infolge immunologischer Prozesse eintretenden Aktivitatsanstiegen streng zu trennen ist In Übereinstimmung mit ASTER und JANDL (16) die ebenfalls bei Fruhmessung einen starken Aktivitatsanstieg beobachteten ließ sich bei Normalpersonen über der Leber nur ein geringer zusatzlicher Aktivitätsanstieg in den ersten Minuten beobachten Die von DAVEY und LANDER (56) mitgeteilten Kurvenverlaufe über das Verhalten markierter Blutplattchen in den ersten Minuten nach Injektion sind nicht verwertbar da bei der von ihnen verwendeten Aufarbeitung mit EDTA eine reversible Sequestration stattfindet. Ein wesentlicher Abbau in der Lunge wie er von einigen Autoren (122) festgestellt wurde konnte nicht beobachtet werden. Aus der Zeit von

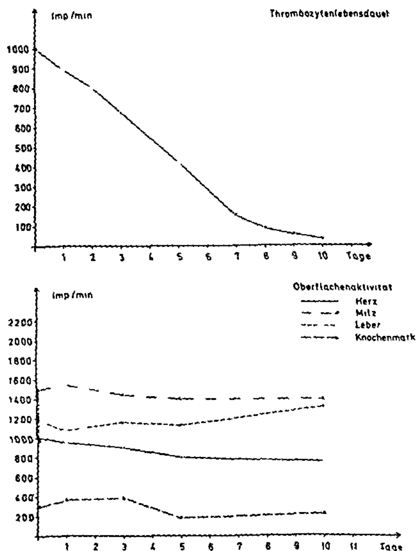


Abb 17 Normaler Verlauf von Thrombozytenlebensdauer und Oberflachenaktivitatswerten bei einem 59 jahrigen Mann.

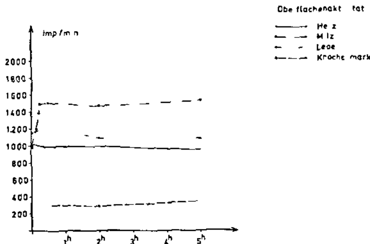


Abb 18 Normales Verhalten der Oberflächenaktivitätswerte in den ersten 5 Stunden (dieselbe Untersuchung wie in Abb 17).

12 bis 17 Minuten in der die bei der Aufarbeitung ladierten Thrombozyten bei Normalpersonen aus dem Kreislauf beseitigt werden, leitet sich andererseits die Schlußfolgerung daß jeder darüber hinaus erfolgende rasche Aktivitätsanstieg in den ersten Stunden Ausdruck einer vermehrten nicht exogen bedingten Thrombozytendestruktion in dem betreffenden Organ ist ohne daß daraus bis jetzt exakte quantitative Rückschlüsse hinsichtlich der absoluten Organradioaktivität möglich sind Der Normalbereich der über Milz und Leber festgestellten Oberflächenaktivitätswerte einschließlich der Mittelwerte ist in Abb 19 und Abb 20 dargestellt Man erkennt daß die Aktivität über der Milz normalerweise höher ist als über der Leber

Der Verlauf der zusätzlichen Aktivitätswerte ist unterschiedlich je nachdem auf welchen Ausgangswert sie bezogen werden Die Messung unmittelbar nach der Injektion der markierten Thrombozyten mit entsprechender Berechnung der zusätzlichen Aktivitätswerte hat sich uns deshalb als wertvoll erwiesen weil eine bei starker Verkürzung der Thrombozytenlebensdauer rapide in den ersten Stunden erfolgende Zerstörung der Blutplättchen nur so erfaßt werden kann. Die derart berechneten zusätzlichen Aktivitätswerte (Abb 21) steigen entsprechend dem beschriebenen, durch Aufarbeitung der Blutplättchen bedingten initialen Aktivitätsanstieg in den ersten 12 bis 17 Minuten vorwiegend über der Milz auf normalerweise nicht über 1000 Impulse/Minute an um dann im weiteren Verlauf nur eine ganz geringe Zunahme aufzuweisen Dieser initiale Aktivitätsanstieg von 1000 zusätzlichen Impulsen/Minute wird nur dann überschritten wenn der Plättchenertrag z.B infolge unsachgemäßer Behandlung beim Aufarbeiten sehr niedrig ist Über der Leber betragen die zusätzlichen Aktivitätswerte während dieses Zeitraumes maximal 100 Imp /min

Bei Berechnung der zusätzlichen Aktivitätswerte unter Zugrundelegung des 30-Minuten-Wertes (Abb 21) verläuft die Kurve vermindert um den initialen Anstieg derjenigen bei Frühmessung grundsätzlich parallel da der erste Wert (30 Minuten Wert) bereits nach erfolgter Ablagerung der geschädigten Thrombozyten gemessen ist Man sieht dann daß die zusätzlichen Aktivitätswerte

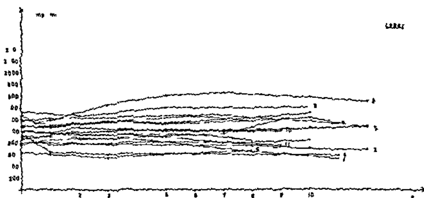


Abb 19 Oberflachenaktivitatswerte uber der Milz bei 14 Normalpersonen.
O O Mittelwert

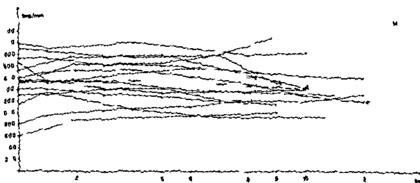
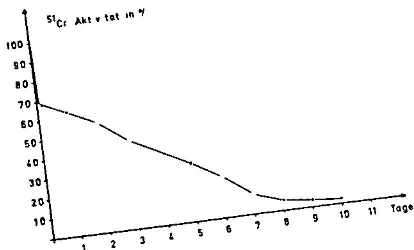


Abb 20 Bereich der Oberflachenaktivitatswerte uber der Leber bei 15 Normalpersonen.
O —————— O Mittelwert

entweder über der Milz mehr als über der Leber oder über Milz und Leber etwa gleichermaßen korrespondierend zu dem darüber dargestellten Abfall der Thrombozytenlebensdauerkurve anstei- gen Bei Normalpersonen haben wir zusätzliche Aktivitätsanstiege über 400 Impulse/Minute vor dem 3 Tag weder über der Milz noch über der Leber beobachtet Nachdem ein relativer Aktivitätsanstieg über einem Organ nur dann zustandekommt wenn es infolge Destruktion von Plättchen zu Aktivitätsansammlungen gekommen ist, können die zusätzlichen Aktivitätswerte nach Ausschluß der initialen durch Aufarbeitung bedingten Sequestration als Zeichen des Thrombozytenabbaues angesehen werden Der Abbau der Thrombozyten erfolgt also normalerweise in Milz und Leber (16,32 55 143)

Die Ermittlung des Abbauortes mit Hilfe der zusätzlichen Aktivitätswerte kann aber im Stich lassen wenn die Thrombozytenlebensdauer extrem kurz ist Die Interpretation der durch Oberflächenaktivitätsmessung gewonnenen Befunde bereitet hinsichtlich einer quantitativen Aussage nach Ansicht aller Autoren, die sich mit Korperaktivitätsmessungen beschäftigt haben im Gegensatz zu den bei Ermittlung des Abbauortes von Erythrozyten gewonnenen Ergebnissen gewisse Schwierigkeiten Die Gründe hierfür sind teilweise methodischer Art Die bei Verwendung von EDTA auftretende initiale Sequestration der Thrombozyten sowie unterschiedlich hohe Plättchenerträge und verschiedenartiger Zeitpunkt der ersten Messung führen zu unterschiedlichen Oberflächenaktivitätswerten Die Hauptschwierigkeit liegt unseres Erachtens aber in der gegenüber der Erythrozytenlebensdauer im allgemeinen geringeren und im speziellen unter Umständen außerordentlich kurzen Thrombozytenlebensdauer Von den verschiedenen Arbeitsgruppen konnte deshalb bisher auch nur eine semiquantitative Aussage über den Abbau der Thrombozyten gemacht werden (16 41 141) Bei Normalpersonen ist die Überlebenszeit ausreichend lange, so daß es zu einem zunehmenden Aktivitätsanstieg kommen kann Ist dagegen die Thrombozytenlebensdauer extrem kurz so kann ein deutlicher zusätzlicher Aktivitätsanstieg selbst bei Messung nach wenigen Minuten ausbleiben Die Lokalisation des Abbaues dieser sehr kurz lebenden Thrombozyten stellt dann den Ort der höchsten Radioaktivität dar Es geht daraus hervor daß die absoluten Oberflächenaktivitätswerte immer insbesondere bei den Fällen mit extrem kurzer Plattchenüberlebenszeit für die Beurteilung des Abbauortes mit herangezogen werden müssen

Von den gemessenen Oberflächenaktivitaten läßt sich allerdings nicht ohne weiteres auf bestimmte Organradioaktivitäten zurückschließen Was die Korrelation zwischen Oberflächenaktivitätswerten und der absolut im Organ befindlichen Radioaktivität angeht, so haben die Untersuchungen von SHULMAN(179) am Phantom gezeigt, daß bei gleicher absoluter Radioaktivitätsmenge in der Milz und in der Leber das Verhältnis der Oberflächenaktivitäten 1,7 1 beträgt. Aus den Abbildungen 22 und 23, in denen die Mittelwerte der Oberflachenaktivitätswerte von Milz und Leber dargestellt sind laßt sich ein Verhaltnis von 1 5 1 entnehmen. Dieser Befund stimmt gut mit den Ergebnissen von SHULMAN überein der ein Milz Leber-Verhältnis der Oberflachenaktivitäten von 1 7 1 bei hamatologisch gesunden Personen fand. Der Abbau der Thrombozyten vollzieht sich also bei Normalpersonen etwa zu gleichen Teilen in der Milz und in der Leber (16,32 143) Die von ASTER und JANDL (16) im Gegensatz zu den Befunden von SHULMAN und unseren eigenen Ergebnissen gemachte Feststellung eines Abbaus von 60 bis 90% der Thrombozyten in der Leber beruht



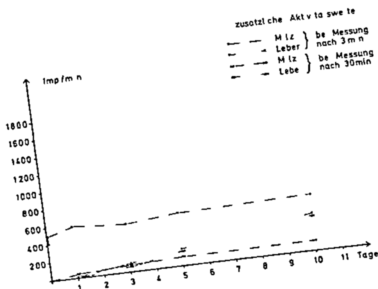


Abb 21 Plattchenertrag und zusatzliche Aktivitatswerte bei einem gesunden Mann
(s. Abb 17 und 18)
Die zusatzlichen Aktivitatswerte sind bezogen auf den 3-min-Wert und den 30-min Wert.

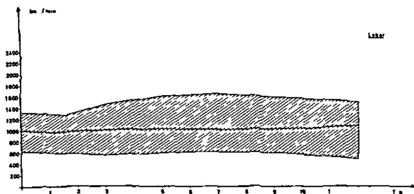
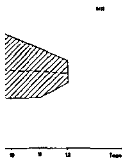


Abb 22 Normalbereich der Oberflachenaktivitatswerte uber der Milz.



e über der Leber

wahrscheinlich auf der Benutzung eines anderen Korrelationsfaktors zur Umrechnung der Oberflachenaktivitat in die wirklich vorhandene Organradioaktivitat Die im Rahmen der Thrombozytenlebensdauerbestimmung durchgeführte Oberflachenaktivitatsmessung zur Ermittlung des Abbauortes erhalt *also insofern ihre begrenzte quantitative* Grundlage, als bei deutlichem Überschreiten der Milz Leber-Oberflachenaktivitat von 1,7 1 ein vorwiegend lienaler Abbau anzunehmen ist Umgekehrt ist ein hepatogener Abbau dann vorhanden wenn der genannte Faktor wesentlich kleiner als 1,7 1 ist Dasselbe gilt grundsatzlich auch für die zusatzlichen Aktivitatswerte hinsichtlich der Interpretation des Abbauortes Nehmen Leber und Milz etwa zu gleichen Teilen an einem vermehrten Abbau teil so bleibt das Verhaltnis 1,7 1 gewahrt Allerdings liegen dann die Oberflachenaktivitatswerte in beiden Organen hoher als bei Normalpersonen aufgezeichnet Als Zeitpunkt der Berechnung der Milz Leber Aktivitat haben wir 24 Stunden nach der Markierung angenommen da insbesondere bei den Fallen mit verkurzter Thrombozytenlebensdauer nach 1 Tag bereits die großte Aktivitatsansammlung in der Leber und der Milz stattgefunden hat

Unter Zugrundelegung dieses Maßstabes laßt sich aus den Oberflachenaktivitatsmessungen semiquantitativ feststellen ob der Abbau lienal hepatogen, vorwiegend bzw rein lienal oder vorwiegend bzw rein hepatogen erfolgt ist

B Idiopathische Thrombozytopenie (ITP)

Die ursprungliche Hypothese einer toxisch bedingten Markhemmung von FRANK (73) aus dem Jahre 1916 über die Aetiologie des als Morbus maculosus haemorrhagicus 1735 von WERLHOF (197) *erst*mals beschriebenen Krankheitsbildes ist erst in den letzten 20 Jahren endgultig verlassen worden Zwar hatte bereits KAZNELSON (103) auf die gesteigerte Zerstorung der Blutplattchen in der Milz hingewiesen Eine gesicherte Theorie uber eine Pathogenese war aber bis 1951 nicht entwickelt worden. Eine entscheidende Wandlung in der Betrachtung des Krankheitsbildes ist erst eingetreten, seitdem HIRSCH *und Mitarbeiter* (39) *sowie* STEFANINI und Mitarbeiter (184) erstmals durch Transfusion von Frischblut unter Beobachtung der Plattchenzahlen feststellen konnten daß die Thrombozyten bei diesen Patienten verkurzt leben Sie stellten Überlebenszeit von wenigen Stunden bei akuten Krankheitsbildern und von wenigen Tagen bei chronischen Formen fest Schließlich konnte HARRINGTON anhand von Übertragungen von Plasma an ITP erkrankter Personen zeigen daß sich im Blut dieser Patienten ein ubertragbarer, plattchenzerstorender Faktor befindet, der zur Thrombozytopenie fuhrt (86)

Nachweis von Antikorpern mit den verschiedenen Methoden (37,86 159,187) sowie morphologische und zahlenmaßige Veranderungen der Megakaryozyten im Knochenmark sind zur Diagnostik und zur Bewertung des Krankheitsbildes herangezogen worden ohne daß sie letzlich eine Klarung herbeifuhren konnten (53 59,142 161) Demgegenuber stellt die Bestimmung der Lebenszeit bei der Zuordnung einer unklaren Thrombozytopenie ein führendes und für die Diagnostik das verlaßlichste und konstanteste Kriterium dar Beim Nachweis von Isoimmunantikorpern ist sie beispielsweise der Komplementfixation weit überlegen, denn mit dieser Methode sind Antikorper erst feststellbar wenn die Überlebenszeit bereits hochgradig verkurzt ist (18,179)

Allerdings ist hier zu sagen daß die idiopathische Thrombozytopenie eine Diagnose darstellt die erst nach Ausschluß anderer Thrombozytopenieformen gestellt werden darf Denn verschiedene auch immunologisch bedingte Formen der thrombozytopenischen Purpura akuter

oder chronischer Verlaufsform werden als idiopathisch bezeichnet, ohne daß sie auf ihre ätiologische und pathogenetische Heterogenität geprüft sind. So müssen denn vorher solche Krankheitsfälle abgetrennt werden, bei denen sich die Thrombozytopenie auf folgende Weise erklären läßt

1 Durch Medikamentenallergie
2 Im Rahmen einer ABO-Unverträglichkeit bei Neugeborenen
3 Durch Isoimmunisierung nach mehrfachen Transfusionen
4 Im Rahmen anderer Immunerkrankungen (z.B Lupus erythematodes)
5 Im Rahmen von lympho-retikulären Erkrankungen
6 Als akute posttransfusionelle Thrombozytopenie
7 Als thrombohaemolytische Thrombozytopenie
8 Im Rahmen einer Sepsis

Bei Anlegen dieser Maßstabe erweist sich die idiopatische Thrombozytopenie als ein relativ seltenes Krankheitsbild Bei 13 Patienten mit einer ITP haben wir 22 Einzeluntersuchungen durchgeführt Über die klinischen hämatologischen und bei der Bestimmung von Überlebenszeit und Destruktionsort gewonnenen Daten gibt die zusammenfassende Tabelle 4 Auskunft

Von klinischen Gesichtspunkten ist die akute Form und der akute Schub einer chronischen idiopathischen Thrombozytopenie von der chronischen Verlaufsform im Interfall abzutrennen

Thrombozytenlebensdauer Plättchenertrag Plättchenertragsfläche

Bei der idiopathischen Thrombozytopenie läßt sich auch im Intervall immer eine mehr oder weniger starke Verkürzung der Überlebenszeit der Blutplättchen feststellen In Abb 24 sind Kurven von 12 Patienten dargestellt Sie zeigen, daß die Lebenszeit wenige Stunden bis einige Tage beträgt. Die Thrombozytenlebensdauer erweist sich am stärksten verkürzt bei den hochakuten Verlaufsformen und dem akuten Schub einer chronischen ITP (Fall 25 29,31) Bei den chronischen Verlaufsformen im Intervall kann die Lebensdauer dagegen einige Tage betragen (Fall 22 24 und 28) wird aber nie normal gefunden Bei 12 Patienten betrug die Lebensdauer zwischen 0 02 und 5,3 Tagen durchschnittlich 0 9 Tage Diese Beobachtungen stimmen mit den Untersuchungen von NAJEAN und Mitarbeitern (142 143) sowie denen von BALDINI und anderen (2, 18 19 43,55) überein die ebenfalls in Abhängigkeit von der Verlaufsform Le-

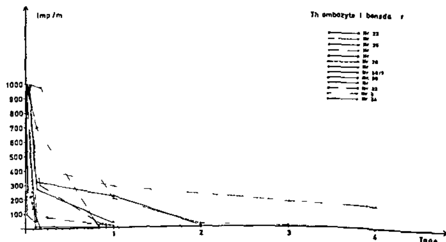


Abb 24 Thrombozytenlebensdauer bei 12 Patienten mit einer ITP
Fall Nr 30 wurde zweimal untersucht (30/1 und 30/2)

benszeiten von wenigen Stunden bis einigen Tagen feststellten ohne aber eine Korrelation zum klinischen Bild herstellen zu können. Demgegenüber sind normale Überlebenszeiten wie sie *COHEN und* GARDNER in einer zweiten, sogenannten nicht immunologischen Form der ITP zusammengefaßt haben (43), von uns in Übereinstimmung mit den genannten Au*toren bei Patienten, die wir der ITP zuge*ordnet haben, nicht beobachtet worden Wahrscheinlich handelt es sich bei diesen von COHEN und Mitarbeitern beobachteten Fällen um ätiologisch anders einzu*ordnende Patienten*

Neben der Unterschiedlichkeit der Lebensdauer bestehen aber bei der ITP auch Differenzen einmal hinsichtlich der Form der Kurve und zum anderen im Hinblick auf den Plättchenertrag. So läßt die Abb 24 deutlich erkennen, daß der Abfall zwar meist linear ist daneben aber insbesondere bei den auf 1 bis 3 Tage verkürzten Lebenszeiten - einen exponentiellen Verlauf annehmen kann Der Einfluß des Plättchenverbrauchs ist hier nicht zu verkennen

Bei der Untersuchung der Thrombozytenlebensdauer ist die Höhe des Plättchenertragswertes bis in die jüngste Zeit nicht *genügend beachtet worden (43) wodurch* Fehldeutungen entstanden sind (19) In der Abb 25 sind die Plättchenertragswerte der in Abb 24 zusammengefaßten Patienten dargestellt Es geht daraus hervor *daß der Anteil der in der Peripherie* befindlichen lebensfähigen Plättchen bei der ITP fast *regelmäßig, möglicherweise in* Abhängigkeit von der Potenz von Plättchenantikörpern vermindert ist Wir fanden bei Fällen unbehandelter ITP Plättchenerträge von 3 7 bis 59 8% Bei den hochakuten Formen ist der Plättchen*ertrag bereits bei Messung nach wenigen* Minuten stark vermindert, so daß anzunehmen ist daß die Masse der Blutplättchen schon zu diesem Zeitpunkt zerstört ist Auf die sich hieraus ergebende bereits oben angedeutete Notwendigkeit der Frühmessung der Oberflächenaktivität und die Korrespondenz der erhobenen Befunde wird später noch eingegangen Daneben finden sich aber auch Formen, bei denen der Plättchenertrag relativ hoch ist, d.h. *in praktisch normale Bereiche fällt mit* allerdings stark verminderter Thrombozytenlebensdauer (Fall 22 28,30,32 und 33) Einerseits können also offenbar die Blutplättchen während der ersten Zirkulation *von Antikörpern erfaßt und zerstört* werden, andererseits aber zunächst größtenteils lebensfähig zirkulieren und dann aber rasch vom Abbau erfaßt werden Ob hier tatsächlich - wie von BALDINI (18) dis*kutiert - differente pathogenetische Me*chanismen wirksam sind ist ein noch offenes Problem Uns scheint aber, daß es sich doch um den Ausdruck der Foudroyanz der Erkrankung handelt d.h im speziellen *der unterschiedlichen im Blut befindlichen* Antikörperaktivität Diese Vermutung wurde mit den Ergebnissen von Untersuchungen mit Isoimmunantikörpern d h gegen die gleichen Plättchenspender gerichteten Antikörper, übereinstimmen Dabei finden sich in Abhängigkeit von der Menge der übertragenen Antikörper reduzierte Plättchenertragswerte (16)

Die bei der Überlebenszeitbestimmung gewonnenen Plättchenertragsflächen lassen bei der ITP sowohl insgesamt als auch bei den einzelnen Krankheitsfällen deutliche Unterschiede erkennen Bei 12 Personen war die Plättchenertragsfläche auf durchschnittlich 4,5 cm^2 gegenüber mindestens 60 cm^2 bei den Kontrollpersonen vermin*dert Als Ausdruck des foudroyanten Ab*baues wiesen die akuten Fälle und die im akuten Schub einer chronischen ITP befindlichen Personen die kleinsten Flächen auf (Abb 26) Eine Korrelation zwischen *den Plättchenertragsflächen und den* Thrombozytenzahlen war nicht festzustellen (Abb 42)

Zusammenfassend ergibt sich also, daß bei der idiopathischen Thrombozytopenie immer verkürzte Überlebenszeiten der Thrombozyten sowie verminderte Plättchenerträge *und Plättchenertragsflächen* gefunden werden Bei akuten Formen und im

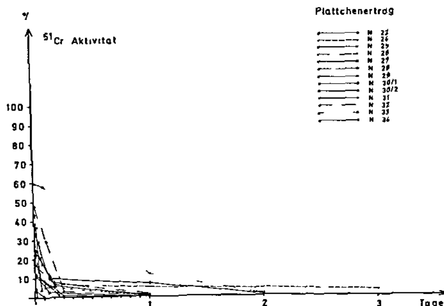


Abb 25 Verhalten des Plattchenertrages bei 12 Patienten mit einer ITP (s. Abb 24)

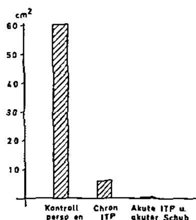


Abb 26 Durchschnittliche Plattchenertragswerte bei 19 Kontrollpersonen, 8 Patienten mit einer chronischen ITP und 3 Patienten mit einer akuten ITP bzw einem akuten Schub einer ITP

akuten Schub einer chronischen ITP lassen sich die stärksten Verminderungen nachweisen Der Plattchenertrag ist bei der akuten Verlaufsform immer extrem bei bei den chronischen Verlaufsformen dagegen meist vermindert Unsere Resultate stimmen mit denen von ASTER und JANDL gut überein (16) Unter Zugrundelegung derselben Parameter ist BALDINI zu den gleichen Ergebnissen gelangt (18)

Abbauort

Die bei der ITP verkurzte Thrombozytenlebensdauer beweist, daß der Abbau sich überstürzt vollzieht Die Lokalisation der vermehrten Destruktion der Thrombozyten laßt sich aus den klinischen Daten insbesondere der Schwere der hamorrhagischen Diathese und dem Grad der Thrombozytopenie nicht von vorneherein erkennen Vielmehr finden sich bei den einzelnen Krankheitsfallen ganz unterschiedliche Abbauformen

Lienaler Abbautyp

Als Beispiel ist der Krankheitsfall eines 24-jahrigen Mannes (Fall Nr 32) mit der chronischen Verlaufsform einer ITP dargestellt Der Patient hatte vor der Untersuchung keine Bluttransfusion erhalten

Die Abb 27 zeigt den Plattchenertrag und das Ergebnis der Bestimmung der zusatzlichen Aktivitätswerte sowie der Oberflachenaktivitätswerte Wahrend über der Leber kaum ein Anstieg zu erkennen ist, nimmt die Aktivitat uber der Milz einen bis zum 1 Tage dauernden steilen Verlauf Die zusatzlichen Aktivitatswerte steigen uber der Milz wie bei Normalpersonen in der ersten Viertelstunde auf etwa 900 Impulse/Min entsprechend einem Abfall des Plattchenertrages auf 48% an Daruber hinaus steigt dann die Aktivität uber der Milz in den nachsten Stunden bis zur Messung am nächsten Tage korrespondierend mit dem praktisch spiegelbildlich verlaufenden Abfall der Thrombozytenlebensdauer auf 3 100 zusatzliche Aktivitätsimpulse pro Minute an, wahrend uber der Leber kein Anstieg zu verzeichnen ist Berechnet man die zusatzlichen Aktivitatswerte nach 30 Minuten, so ergeben sich die in Abb 27 speziell markierten Kurven Man sieht, daß sie den bei Fruhmessung festgestellten Werten vermindert um den initialen Aktivitatsanstieg grundsatzlich parallel verlaufen Der gegenüber Normalpersonen hohe Ausgangswert von 850 zusatzlichen Impulsen pro Minute weist darauf hin daß bereits in den ersten beiden Stunden ein starker Thrombozytenabbau in der Milz erfolgt ist Daneben laßt sich ein bei Überlebenszeiten von einigen Stunden bis wenigen Tagen weiterer Aktivitätsanstieg uber der Milz feststellen Auch das nach 24 Stunden gemessene Verhaltnis von Milz- zu Leberoberflachenaktivitat von 5 8 gegenuber 1,5 bei Normalpersonen ist ein Beweis für die starke Aktivitatsansammlung in der Milz Es handelt sich hier um die typische Kurve eines lienalen Abbaues

In der Abb 28 ist ein weiteres Beispiel für für einen rein lienalen Abbautyp aufgezeichnet Es zeigt sich prinzipiell dasselbe Bild wie in Abb 27 Entsprechend einem raschen Abfall der Thrombozytenlebensdauerkurve steigen die Oberflachenaktivitatswerte uber der Milz stark an wahrend sie uber der Leber kaum eine Änderung erfahren Die zusatzlichen Aktivitatswerte weisen bereits nach 4 Stunden über der Milz einen hohen Wert von 810 Impulsen auf Bereits bis zu diesem Zeitpunkt ist also ein erheblicher Abbau von Thrombozyten in der Milz erfolgt Korrespondierend mit dem weiteren Abfall der Lebensdauerkurve steigen die zusatzlichen Aktivitatswerte uber der Milz noch bis zum nächsten Tag an Nach 24 Stunden hat sich uber der Milz gegenuber der Leber eine große Aktivitat aufgebaut, die sich in dem gegenüber Normalpersonen hohen Faktor von 6 8 ausdruckt

Der genannte hohe Oberflachenaktivitatswert uber der Milz laßt sich als regelhafte Erscheinung darstellen wenn man die durchschnittlichen Aktivitatswerte der Pa

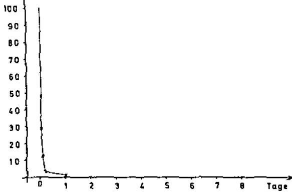
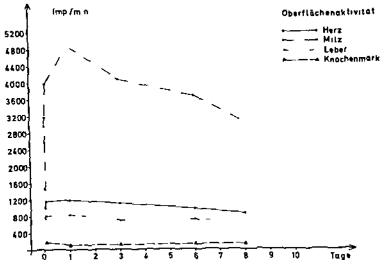


Abb 27 Plattchenertrag, Oberflachenaktivitatswerte und zusatzliche Aktivitatswerte bei einem 24-jahrigen Mann mit einer chronischen ITP Rein lienaler Abbau.



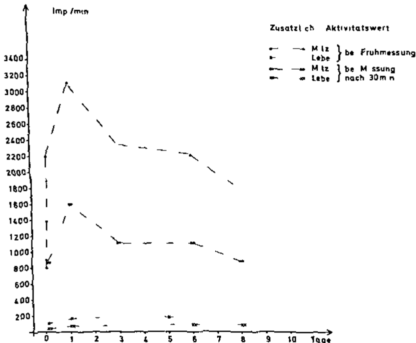
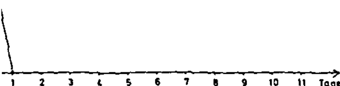
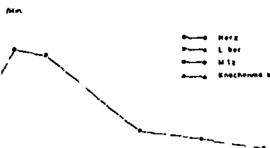


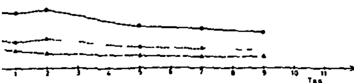
Abb 28 Lienaler Abbautyp bei einer 61-jährigen Patientin mit chron. ITP (Fall Nr 27)

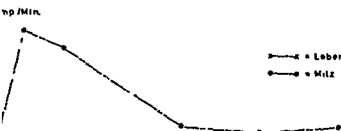
Thrombozytenlebensdauer



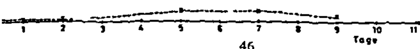


Oberflächenaktivität





Zusätzliche Aktivitätswerte



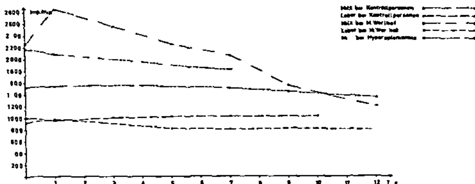


Abb 29 Mittelwerte der Oberflächenaktivitäten der Milz und der Leber bei Kontrollpersonen, der Milz bei idiopathischer Thrombozytopenie und bei Hypersplenismus und der Leber bei der chronischen Verlaufsform der ITP

tienten mit idiopathischer Thrombozytopenie mit denen bei Kontrollpersonen vergleicht wie es in Abb 29 geschehen ist Man sieht daß die Oberflächenaktivitätswerte bei den Kontrollpersonen durchschnittlich um 1 500 Impulse pro Minute liegen während bei den Patienten mit einer ITP bereits die Ausgangswerte 2 200 Impulse pro Minute betragen und dann auch noch einen Anstieg bis zum nächsten Tag auf 2 900 Impulse d.h etwa das Doppelte der Norm aufweisen Klammert man die Patienten mit dem später noch zu besprechenden hepatogenen Abbau aus so errechnet sich eine durchschnittliche Oberflächenaktivität über der Leber nach 24 Stunden von 1 000 Impulsen pro Minute Daraus ergibt sich das gegenüber hämatologisch gesunden Personen hohe Milz zu Leber Aktivitätsverhältnis von 2 9 1 0

Die hohe Aktivitätsansammlung in der Milz läßt sich darüberhinaus szintigraphisch festhalten Wie das Szintigramm eines Patienten mit einer ITP (Abb 30, Fall Nr 30) zeigt ist aufgrund der hohen Radioaktivitätsansammlung in der Milz eine Darstellung und Abgrenzung des Organs möglich. Man kann daraus weiterhin ableiten daß die immunologische Aktivität unabhängig von der Größe des Organs ist Soweit uns das Weltschrifttum zugänglich war handelt es sich hier übrigens um die erste szintigraphische Darstellung der Milz mit ^{51}Cr markierten Thrombozyten im Rahmen einer Thrombozytenlebensdauerbestimmung. Während früher aufgrund von Messungen der Thrombozytenzahlen im Arterien- und Venenblut der Milz eine Destruktion der Blutplättchen bei der ITP in diesem Organ verneint wurde (184) ist durch die dargelegten Befunde bewiesen daß in einem Teil der Fälle von ITP der Abbau ganz vorwiegend oder rein lienal erfolgt.

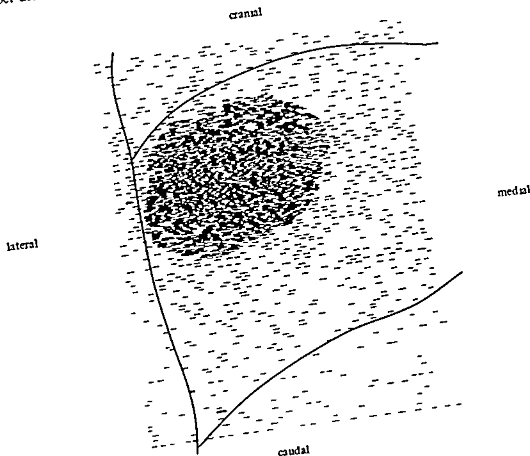
Aus den Abbildungen 27 und 28 sind die Charakteristika des lienalen Abbautyps der idiopathischen Thrombozytopenie zu entnehmen

Hohe Oberflächenaktivitätswerte über der Milz die das bei Normalpersonen vorhandene Verhältnis von 1,5 gegenüber der Leberaktivität weit überschreiten starker über die erste Viertelstunde andauernder zusätzlicher Aktivitätsanstieg über diesem Organ der korrespondierend zu dem Abfall der Thrombozytenlebensdauer ist. Beträgt die Lebensdauer etwa 20 Stunden bis wenige Tage so findet sich auch während dieser Zeit ein weiterer zusätzlicher Aktivitätsanstieg. Bei 11 Patienten mit chronischer Verlaufsform wurde 6 mal ein

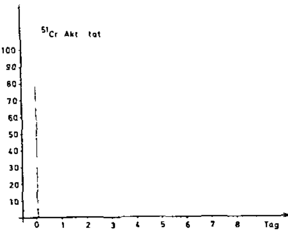
rein lienaler Abbautyp festgestellt 2 mal war die Milz vorwiegender Abbauort, einmal waren Milz und Leber gleichermaßen an der vermehrten Zerstörung der Blutplättchen beteiligt

Hepatogener Abbautyp

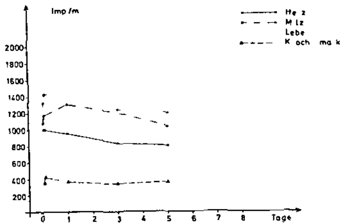
Beispielhaft für diese Abbauform sind in Abb 31 die Ergebnisse von Thrombozytenlebensdauer und Oberflächenmessungen aufgezeichnet Bereits wenige Minuten nach der Injektion werden vor allem über der Leber gegenüber der Norm hohe Oberflächenaktivitätswerte die höher als die über der Milz liegen festgestellt Der Plättchenertrag von nur 4 7% gibt Auskunft, daß zu diesem Zeitpunkt bereits etwa 95% der Thrombozyten den Kreislauf verlassen haben und erklärt diesen hohen Aktivitätswert über der Leber Entsprechend dem Abfall während der ersten halben Stunde tritt dann ein nur noch relativ geringer Anstieg über der Leber auf Die zusätzlichen Aktivitätswerte über der Leber betragen hier immerhin noch das Vierfache der bei Normalpersonen gemessenen Werte Betrachtet man das Milz- zu Leber Aktivitätsverhältnis von 0,9, so wird klar, daß es sich hier um einen rein hepatogenen Abbau handelt



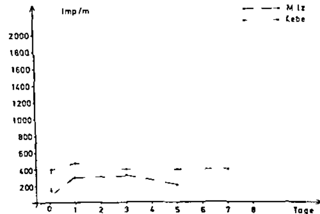
…szintigramm des Falles Nr 30 in Bauchlage



Plattchenertrag



Oberflachenaktivitat



Zusatzliche Aktivitatswerte

Abb 31 46-jähriger Mann mit schwerem akuten Schub einer ITP (Fall Nr 31) Darstellung von Plattchenertrag, Oberflachenaktivitat und zusatzlichen Aktivitatswerten. Hepatogener Abbau.

Der im akuten Schub einer chronischen ITP und bei den akuten Verlaufsformen infolge fast vollständiger Zerstörung der Blutplättchen während der ersten Zirkulation niedrige Plättchenertragswert macht verständlich, daß ein weiterer wesentlicher Aktivitätsanstieg grundsätzlich nicht zu erwarten ist, so daß ein weiterer Anstieg der Oberflächenaktivität und dementsprechend auch der zusätzlichen Aktivitätswerte dann nicht mehr festgestellt werden kann, wenn die erste Messung erst nach 30 Minuten erfolgt Denn ein weiterer Aktivitätsanstieg kann nur dann eintreten, wenn noch mit ^{51}Cr beladene Blutplättchen zerstort werden Dadurch entsteht die bereits diskutierte Hauptschwierigkeit in der Interpretation der zusätzlichen Aktivitätswerte bei sehr raschem Abbau der Blutplättchen Wenn die Thrombozyten zur Zeit der ersten Messung bereits vollständig zerstort sind und die Herzaktivitat schneller als die der übrigen Organe abfällt, resultiert ein pseudonormaler Verlauf Die in diesen Fallen allerdings immer feststellbaren hohen Oberflächenaktivitätswerte uber der Leber decken dann in Zusammenhang mit der extrem kurzen Lebensdauer diesen Fehler auf

Sowohl bei einer akuten Verlaufsform (Fall 29) als auch bei 2 schweren akuten Schüben einer chronischen idiopathischen Thrombozytopenie (Fall Nr 25 und 31) ließ sich im Gegensatz zur chronischen Verlaufsform dieser Befund namlich die hohen Oberflächenaktivitatswerte, über der Leber feststellen Die Abb 32 zeigt die Mittelwerte der Oberflachenaktivitatswerte bei den 3 Patienten Sie liegen ein deutig höher als die bei Kontrollpersonen und bei chronischen Verlaufsformen Neben der Höhe bestätigt auch das in Tabelle 4 einzeln aufgeführte Milz zu Leber-Oberflächenaktivitätsverhältnis das nie über 1 lag, daß die großte Aktivitatsansammlung als Ausdruck der Thrombozytendestruk tion bei diesen Fällen in der Leber zu finden war ASTER und JANDL haben bei der Untersuchung eines Falles von akuter ITP identische Befunde erhoben (16)

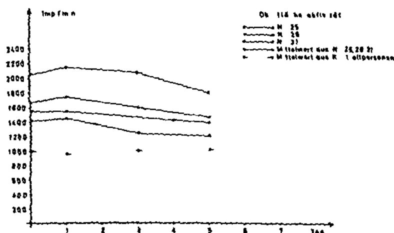


Abb 32 Leber-Oberflächenaktivitätswerte von einem Patienten mit einer akuten ITP (Fall Nr 29) und 2 Patienten mit einem schweren, akuten Schub einer ITP im Vergleich zur durchschnittlichen Aktivität über der Leber bei Normalpersonen.

Initial hohe Oberflächenaktivitätswerte über der Leber, die die der Milz oft überschreiten, bei extrem erniedrigtem Plättchenertrag und geringer weiterer zusätzlicher Aktivitätsanstieg über Leber und Milz sind dementsprechend die Kennzeichen des hepatogenen Abbautyps

Zwischen der ausschließlichen Destruktion in der Milz und dem hepatogenen Abbautyp finden sich Übergänge Die Abb 33 zeigt ein Bild eines vermehrten kombinierten hepato-lienalen Abbautyps mit hohen zusätzlichen Aktivitätswerten über der Leber die aber durch einen weiteren Anstieg der zusätzlichen Aktivitätswerte über der Milz noch überschritten werden Die Anstiegszeit und das Erreichen des Plateaus stimmen mit dem Verlauf der Plättchenertragskurve überein Das scheinbar normale Verhältnis von Milz zu Leber-Oberflächenaktivität von 1 8 ist durch einen gleichzeitigen Anstieg der Oberflächenaktivität über diesen beiden Organen bedingt (s Tab 4) Bei Vergleich der klinischen und haematologischen Daten mit den bei der radioaktiven Markierung gewonnenen Resultaten kann man mit NAJEAN (141) sagen, daß eine Beziehung zwischen Abbauort und Länge der Erkrankung dem Ausmaß der Thrombopenie und dem Vorhandensein von Antikörpern nicht besteht

Veränderungen der Abbauform und des Destruktionsortes unter der Therapie

---

Der Erfolg der Therapie bei der akuten und chronischen idiopathischen Thrombopenie, für die heute im wesentlichen außer Nebennierenrindenhormonen und Antimetaboliten die Splenektomie zur Verfügung steht (83 112) ist im einzelnen nicht vorherzusagen Spontan oder durch Medikamente kann es zu einer Normalisierung aller Werte kommen Daneben kann die medikamentöse Behandlung eine unvollständige Remission herbeiführen oder sogar ineffektiv sein (152)

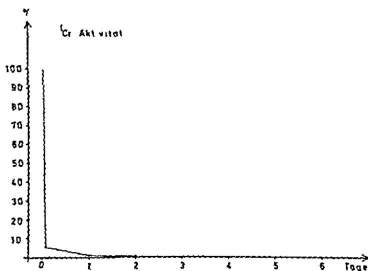
Als Beispiel einer vollständig spontanen Ausheilung sind die Thrombozytenlebensdauer und die Oberflächenaktivitätswerte einer akuten Verlaufsform (Fall 29) in Abb 34 dargestellt Der im akuten Stadium auf 3,7% erniedrigte Plättchenertrag und die auf 0,2 Tage verkürzte Thrombozytenlebensdauer steigen nach Ausheilung auf normale Werte von 58 8% bzw 8 8 Tage an Der im akuten Stadium an der hohen Oberflächenaktivität über der Leber (Milz - zu Leber - Aktivitätsverhältnis 1 0) erkennbare vermehrte Abbau in der Leber wandelt sich in einen vorwiegend lienalen Abbautyp um

Mit Prednisolon läßt sich oft eine vollständige Normalisierung der Thrombozytenlebensdauer erreichen Abb 35 zeigt beispielhaft die Ergebnisse bei einer 42-jährigen Frau mit einer idiopathischen Thrombozytopenie Vor der Behandlung betrug die Thrombozytenlebensdauer 5,3 Tage mit einem vorwiegend lienalen Abbautyp Nach Behandlung mit Prednisolon stieg die Thrombozytenzahl von 6 250 auf 244 000 pro mm^3 an Der Abbau der mit 9 1 Tagen normal lebenden Blutplättchen vollzog sich jetzt wie bei Normalpersonen zu gleichen Teilen in der Milz und in der Leber wie das aus dem Abfall des Milz zu Leber Aktivitätsverhältnisses von 2,3 auf 1 4 hervorgeht. Die Corticoidtherapie hat offenbar zu einer Verhinderung des Abbaues in der Milz geführt.

Als Beispiel einer unvollständigen Besserung nach Prednisolon ist der in Abb 36 aufgezeigte Fall (Nr 25) dargestellt Im akuten Schub in dem keine Frühmessung durchgeführt wurde betrug die Thrombozytenlebensdauer 2 5 Stunden und der Abbau erfolgte, wie die hohen Aktivitätswerte über der Leber und das Milz zu Leber Aktivitätsverhältnis von 0,95 erkennen lassen hepatogen

Nach Prednisolonbehandlung verlängerte sich die Überlebenszeit der Thrombozyten auf 3,5 Tage Die zusätzlichen Aktivitätswerte über der Milz steigen dementspre

chend bis zum 5 Tage auf ein Maximum an Wie bei dem bereits gezeigten Beispiel (Abb 31) einer akuten Verlaufsform der ITP ist es hier allerdings unter Prednisolon zu einer Änderung des Abbauortes gekommen Der im akuten Schub hepatogene Abbautyp hat sich in einen lienalen verwandelt



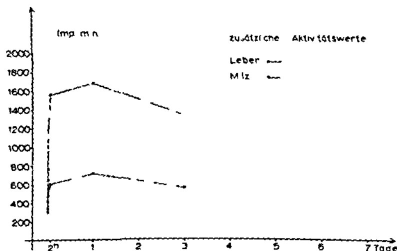
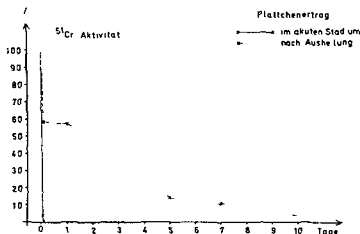


Abb 33 27-jähriger Mann mit mäßig schwerem Schub einer ITP (Fall Nr 33) Thrombozytenlebensdauer und zusätzliche Aktivitätswerte Kombinierter hepato-lienaler Abbau.



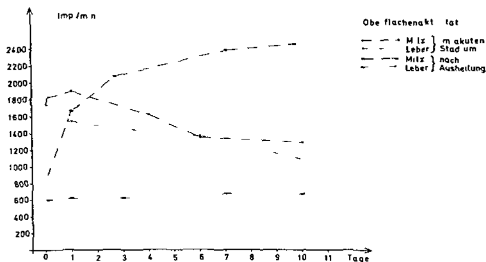


Abb 34 34-jährige Frau mit einer akuten ITP (Fall Nr 29) Verhalten des Plattchenertrages und der Oberflachenaktivitatswerte uber der Milz und uber der Leber im akuten Stadium und nach Ausheilung.

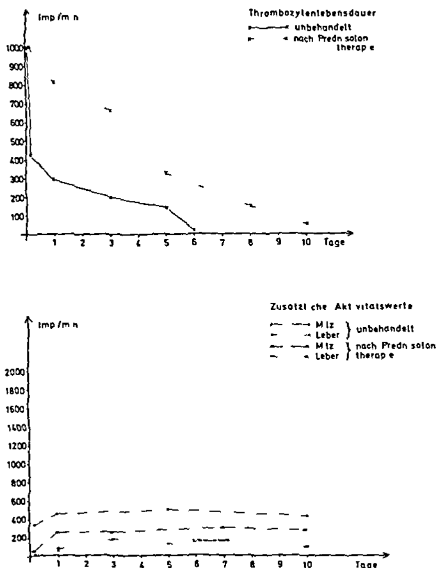
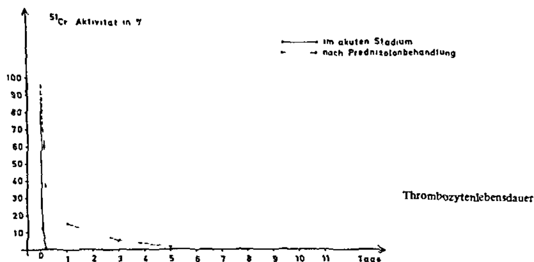


Abb 35 Thrombozytenlebensdauer und zusätzliche Aktivitätswerte vor und nach Behandlung bei einer 42-jährigen Frau mit einer chronischen ITP (Fall 24) Kortikoid induzierte Normalisierung von Überlebenszeit und Abbau.



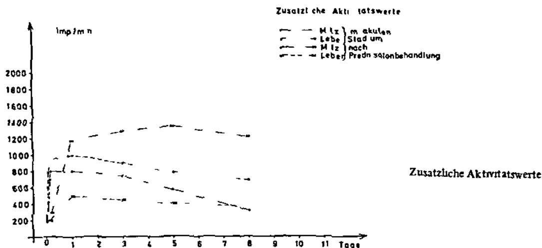


Abb 36 Fall Nr 25 Chron. ITP im akuten Schub und nach Prednisolonbehandlung. Unvollstandige Remission auf Kortikoide.

Die Splenektomie kann zu einer vollständigen Ausheilung führen. Der in Abbildung 37 dargestellte Fall Nr 32 zeigt das. Vor der Splenektomie betrug die Thrombozytenlebensdauer 4 Stunden. Wie man sieht, ist entsprechend dem hohen korrespondierend zum Abfall der Thrombozytenlebensdauer erfolgenden Anstieg uber der Milz der Abbau rein lienal Nach der Splenektomie ist die Thrombozytenlebensdauer normalisiert und der Abbau der normal lebenden Thrombozyten erfolgt - wie es der zunächst schnelle, dann kontinuierliche Anstieg der zusatzlichen Aktivitatswerte uber der Leber zeigt - hepatogen Das RES übernimmt, wie wir es bei entsprechenden Fallen gesehen haben (s Abb 50) den normalerweise zu etwa gleichen Teilen in Milz und Leber erfolgenden Abbau Dabei erreicht die Leberaktivität Werte, die etwa in der gleichen Hohe wie die Milzaktivität bei Normalpersonen liegen Die Entfernung der Milz bewirkt also über die Beseitigung des Destruktionsortes eine Normalisierung der Thrombozytenlebensdauer

Die aufgezeichneten Befunde lassen die Frage aufkommen wie der Abbau der Thrombozyten erfolgt. Bei der Diskussion dieser Frage ist man geneigt allgemein bekannte pathogenetische Prinzipien zum Vergleich heranzuziehen Immerhin laßt der rapide Abbau der Thrombozyten bei der ITP in Analogie zu den hämolytischen *Anämien an einen intravaskulären* Zerfall der Thrombozyten denken Nachdem bekannt ist, daß freies ^{51}Cr sehr schnell im Urin ausgeschieden wird (124) und bei Verwendung unserer Technik nur Spuren ungebundener Chrommengen vorhanden sind wäre zu erwarten gewesen daß die ^{51}Cr Ausscheidung im Urin in den ersten 24 Stunden bei einem intravaskulären Zerfall wesentlich hoher als die bei Normalpersonen sein wurde Die Tabelle 5 zeigt daß die ^{51}Cr Ausscheidung bei 4 Kontrollpersonen in den ersten 24 Stunden durchschnittlich 4 05% betrug. Die bei 4 Patienten mit einer ITP und einer Patientin mit einem Lupus erythematodes ermittelte Urinausscheidung in den ersten 24 Stunden war mit 4,9% praktisch identisch Die von AAS und GARDNER beobachteten hohen Urinaktivitaten von 25 bis 45% auch bei Normalpersonen lassen sehr vermuten, daß sie durch eine unvollständige Beseitigung ungebundener Radioaktivitat bei der Markierung der Thrombozyten zustande gekommen sind. Die tagliche Radioaktivitätsausscheidung bei einem Patienten mit einer ITP und einer Kontrollperson ist im einzelnen nochmals in Abb 38 aufgetragen. Es geht daraus hervor, daß wesentliche Unterschiede nicht bestehen und daß die Ausscheidung von ^{51}Cr zu keiner Zeit 6% der injizierten Aktivitat uberschritt. Aus den Urinaktivitätswerten lassen sich dementsprechend keine Hinweise für einen intravasalen Abbau erblicken.

Prinzipiell besteht natürlich die Moglichkeit daß eine gegenuber Normalpersonen erhohte Urinaktivitat dadurch verhindert wird daß sich die Radioaktivität bei Zerfall der Thrombozyten an Substanzen bindet, die für das Nierenfilter nicht passierbar sind Bei Vergleich des nach einstundiger Zentrifugation bei 18 000 U/min vollig thrombozytenfreien Plasmas zeigt sich zwischen den Werten bei 3 Kontrollpersonen mit denen von 4 Patienten mit ITP im Durchschnitt kein Unterschied (Tabelle 6) Gegenuber den Normalpersonen liegt also bei den Patienten mit idiopathischer Thrombozytopenie keine *erhohte freie Plasmaradioaktivität* vor

Daruberhinaus war in der operativ entfernten Milz von 2 Patienten mit dem lienalen Abbautyp einer ITP rund 80% der injizierten Aktivitat nachweisbar Entsprechende Ergebnisse haben NAJEAN und Mitarbeiter mitgeteilt Sie fanden bei hepato-lienalem Abbau 5 30%, bei lienalem Abbau 40 80% der injizierten Aktivität in der Milz (142)

Diese Befunde, die ganz eindeutig gegen einen intravasalen Abbau der Thrombozyten sprechen stimmen überein mit den von CRONKITE und Mitarbeitern (48 49 50) durchgeführten Untersuchungen von

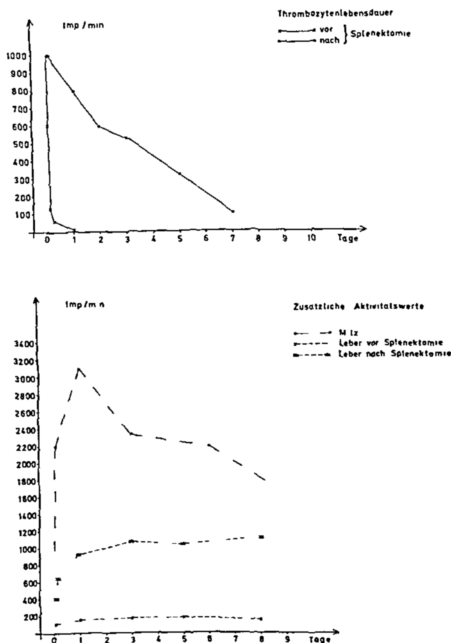


Abb 37 Thrombozytenlebensdauer und zusätzliche Aktivitätswerte vor und nach Splenektomie bei einem 24-jährigen Mann mit einer chronischen ITP mit rein lienalem Abbau (Fall Nr 32) Normalisierung der Plättchenüberlebenszeit und Verlagerung des Abbaus in die Leber

Fall-Nr	Diagnose	^{51}Cr-Ausscheidung i Urin i % der injiz Dosis
29	Idiopathische Thrombozytopenie (akut Stad)	3 o
31	Idiopathische Thrombozytopenie	5 3
32	Idiopathische Thrombozytopenie	4 8
33	Idiopathische Thrombozytopenie	5 3
44	Lupus erythematodes	6 3
	Mittelwert	4 9 (3 o - 6 3)

Fall-Nr	Diagnose	^{51}Cr-Ausscheidung i Urin i % der injiz Dosis
29	Idiopathische Thrombozytopenie nach Ausheilung	6 o
2o	Zustand n CO Vergiftung	3 1
14	Vegetative Dystonie	2 8
19	Schwere Osteochondrose	4 3
	Mittelwert	4 o5 (2 8 - 6 o)

Tab 5 ^{51}Cr Ausscheidung im Urin bei 4 Patienten mit einer ITP sowie einer Patientin mit einem Lupus erythematodes sowie 4 Kontrollpersonen.

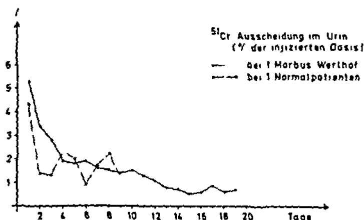


Abb 38 Tägliche ^{51}Cr Ausscheidung im Urin bei einem Patienten mit einer ITP (Morbus Werlhof) und einer Kontrollperson.

mit $Na_2S^{35}O_4$ radioaktiv markierten Plättchen an thrombozytopenischen Tieren. Dabei ließen sich autoradiographisch Blutplattchen im Bereich des Endothel vorwiegend von Milz und Leber feststellen dagegen nicht im Bereich der großen Gefäße Für den Menschen liegen Untersuchungen zur Frage des intravasalen Abbaues bisher nicht vor Zwar haben MAUPIN sowie JULLIARD (101) bereits 1951 Befunde von Organradioaktivitätsmessungen nach Übertragungen von markierten menschlichen Thrombozyten auf Tiere vorgelegt, doch können diese Ergebnisse selbstverständlich nicht auf die Verhältnisse beim Menschen übertragen werden Der Abbau der Thrombozyten erfolgt also nicht intravasal sondern organgebunden in Milz und Leber

Plasma ^{51}Cr-Gehalt n ch 1 stündige Zentrif g tion be 18 ooo U /min (% der injizierten Do is)

bei 3 No malpati nten			bei 3 Patienten mit id opath Thrombozytopeni und 1 Patient mit Lupu rythematod		
Fall Nr	**Zeit n h d Injektion**	**Plasm ^{51}C -Gehalt (% der injizi rten Dosis)**	**Fall Nr**	**Z t na h d Injektion**	**Plasma ^{51}Cr-Gehalt (% d inji iert n Do)**
13	5 min	o 42	3o	5 min	o 62
	35 min	o 34		6o m n	o, 34
	2 Std.	o 73	33	1o min	2, 4
	1 Tag	o 32		3o min	1 8
	4 Tage	o 26		2 Std	1 1
	6 Tage	o 22		1 Tag	o, 48
	9 Tage	o 16		2 T ge	o 5
	1o Tage	o 18	44	5 min	o 29
	11 Tage	o 19		3o min	o 42
14	5 min.	2 o9		2 Std	o 33
	3o min	1 48		1 T g	o, 54
	2 Std	2 o5	34	5 min	o, 34
	1 Tag	1 o5		3o min	o 26
	3 T ge	o 37		2 Std	o 23
	6 Tage	o 29		1 T g	o, 28
	7 Tage	o, 27			
	8 Tage	o 33			
	1o T ge	o 35			
15	3o min	o 5			
	2 Std.	1 6o			
	4 Tage	o, 55			
	5 Tage	o 43			
	7 Tage	o 34			

Tab 6 Plasma-^{51}Cr-Gehalt bei 3 Normalpersonen und 3 Patienten mit ITP sowie 1 Patienten mit Lupus erythematodes.

Bemerkungen zur Immunpathogenese der idiopathischen Thrombozytopenie

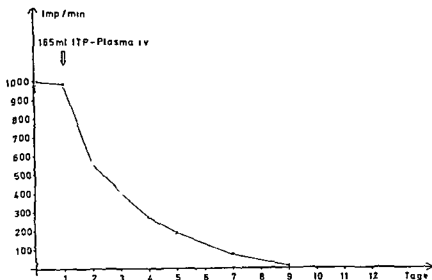
Seit HARRINGTON s heroischem Selbstübertragungsversuch von ITP Plasma besteht kein Zweifel mehr, daß sich bei der idiopathischen Thrombozytopenie im Blut ein übertragbarer plattchenzerstörender Faktor befindet. Inzwischen ist es SHULMAN (179) gelungen, diesen Faktor naher zu charakterisieren Er befindet sich in der 7-S-Gammaglobulinfraktion ist speziesspezifisch, wird von Thrombozyten absorbiert, reagiert mit autologen und homologen Blutplättchen und ist qualitativ und quantitativ den Isoimmunantikorpern vergleichbar In ahnlicher Weise konnten wir auch durch Übertragungsversuche von Plasma eines Patienten (Fall Nr 32) eine dosisabhangige Verkürzung der Lebenszeit der Thrombozyten feststellen Die Abbildungen 39 und 40 zeigen daß es nach Übertragung des Plasmas auf Empfänger zu einem starken Radioaktivitatsanstieg als Ausdruck der Destruktion der Thrombozyten kommt Zur selben Zeit findet ein Abfall der Thrombozytenlebensdauer mit entsprechender Verkurzung der Überlebenszeit statt.

Bei Durchsicht unserer eigenen Befunde (siehe Tabelle 4) erkennt man daß sich die Zerstorung der Thrombozyten bei den Patienten mit der akuten Verlaufsform und solchen mit schweren akuten Schuben einer chronischen ITP (Fall 25,29 und 31) in der Leber vollzieht. Demgegenuber ist bei den Patienten mit der chronischen Verlaufsform der ITP (Fall 22 24 26 27 28 30,32 und 34) die Milz Ort der vermehrten Destruktion Einen ahnlichen Befund haben ASTER und JANDL (16) vorgelegt Bei einem 64-jahrigen Mann mit einer akuten ITP war die Abbauform mit dem in Abb 31 dargestellten Verlauf praktisch identisch Auch SHULMAN (179) fand bei 3 Patienten mit extrem kurzer Thrombozytenlebensdauer (2 Patienten mit einer ITP und 1 Patient mit einer Isoimmunisierung durch mehrfache Transfusionen) außerordentlich hohe Oberflachenaktivitatswerte über der Leber. Weiterhin fällt auf daß nach Übergang der akuten Thrombozytopenie in Ausheilung (Fall 29) bzw bei Auftreten einer Remission (Fall 25 und 34) der Abbauort von der Leber zur Milz wechselt Dieser Wandel des Destruktionsortes ist unter Beachtung der Tatsache, daß es sich dabei um eine semiquantitative Aussage handelt, erkenntlich an der Veranderung des Milz zu Leber-Oberflachenaktivitätsverhaltnisses (s Tab 4)

Übertragungen mittels Komplementfixation quantitativ erfaßbarer thrombozytarer Isoimmunantikorper auf markierte Personen lassen erkennen, daß kleine Mengen des Antikorpers zu einer Zerstörung der Empfängerthrombozyten in der Milz führen, wahrend große Mengen desselben Antikorpers einen hepatogenen Abbau zur Folge haben (16) SHULMAN kam bei Transfusionen von ITP Plasmen zu grundsatzlich gleichen Resultaten Bei kleinen Mengen ITP Plasma lag der Abbauort in der Milz, bei großen in der Leber (179)

Unsere eigenen Befunde lassen sich mit diesen genannten experimentellen Ergebnissen von ASTER und JANDL, SHULMAN sowie BALDINI (18) in Einklang bringen wenn man annimmt daß der Abbau der Thrombozyten bei der ITP offenbar in Abhängigkeit von der Potenz der Antikorper erfolgt so daß die Thrombozyten bei starker Sensibilisierung vorzugsweise in der Leber und bei schwacherer vorwiegend in der Milz zerstort werden Dazu passen die tierexperimentellen Befunde von BALDINI (18) Wurde die Überlebenszeit der Thrombozyten durch Transfusion von Isoimmunantikörpern auf nicht meßbare Werte herabgesetzt so war die großte Radioaktivitätsmenge in der Leber zu finden Dieses Verhalten ist nicht überraschend und entspricht dem Abbautyp von durch Isoimmunantikorper geschädigten Erythrozyten (100)

Retrospektiv erhalt die dem Kliniker bekannte Tatsache der hohen Operationsmortalitat der akuten ITP durch diese



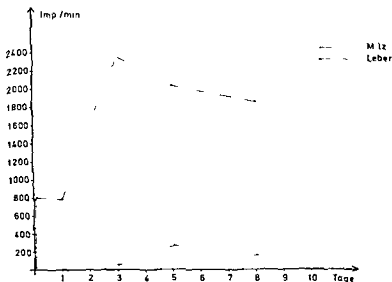
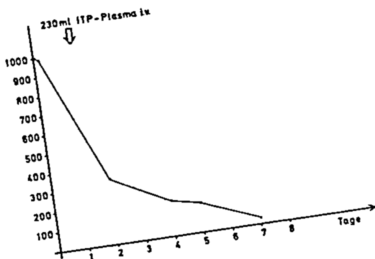


Abb 39 Thrombozytenlebensdauer und zusatzliche Aktivitatswerte uber Milz und Leber nach Transfusion von 165 mL ITP Plasma des Falles Nr 32



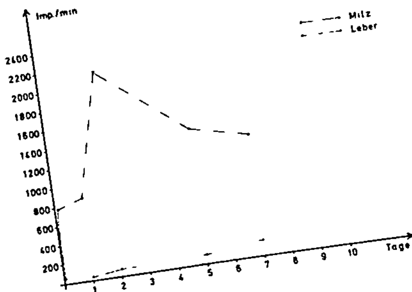


Abb 40· Transfusion von 230 ml ITP Plasma des Falles Nr 32 auf eine Kontrollperson. Darstellung der Thrombozytenlebensdauer und der zusätzlichen Aktivitätswerte über der Milz und über der Leber

Ergebnisse insofern ihre pathophysiologische Erklärung, als bei dieser Verlaufsform mit ihrem hepatischen Abbautyp der Ort der vermehrten Destruktion unberührt bleibt. Das Operationsrisiko wird noch dadurch vergrößert, daß bei der akuten Form der ITP infolge der rasanten Plättchenzerstörung von einer Thrombozytentransfusion kein Erfolg erwartet werden kann.

Der Abbau selbst ist offensichtlich an das retikulo-endotheliale System gebunden. Für diese Annahme spricht einmal, daß ein intravaskulärer Zerfall der Thrombozyten nicht stattfindet. Die Übertragung von ITP-Plasma auf Personen mit einer Milz führt zu einer wesentlich schnelleren Zerstörung der Thrombozyten als bei Splenektomierten, bei denen mit der Milz ein großer Teil des RES entfernt worden ist (86). Schließlich konnte SHULMAN nachweisen, daß die Thrombozyten bei Trägern einer kongenitalen Sphärozytose durch ITP-Plasma in wesentlich geringerem Grade zerstört werden als bei Normalpersonen. Er leitete hieraus die Hypothese ab, daß das RES für den Thrombozytenabbau durch den vermehrten Anfall von Erythrozyten blockiert sei. Diese Überlegung ließ sich von ihm insofern bestätigen, als die Reaktion der Thrombozyten auf ITP-Plasma nach experimenteller Blockade des RES mittels Erythrozytenstroma vermindert war (180).

Die Nebennierenrindenhormone führen bei Normalpersonen nicht zu einer Verlängerung der Thrombozytenlebensdauer. Ihre Wirkung bei der idiopathischen Thrombozytopenie ist eine zweifache. Die bei den Fällen 22, 25, 33 und 44 gemessenen Oberflächenaktivitätswerte (s. Tab. 4) lassen erkennen, daß mit klinischer Besserung unter Corticoidtherapie der Abbauort vorwiegend oder ganz in die Milz verlagert wird. Die Annahme einer Einwirkung der Nebennierenrindenhormone auf den Abbauort wird durch Daten von SHULMAN belegt. Er stellte fest, daß Nebennierenrindenhormone den Abbau von Thrombozyten nach Transfusion kleiner Mengen ITP-Plasmas verhindern (180).

Die klinisch bekannte Tatsache, daß die Corticoidtherapie manchmal auch nach erfolgloser Splenektomie bei der ITP eine Wirkung erzielen kann (18), spricht zusammen mit unseren Befunden dafür, daß die Nebennierenrindenhormone die Zerstörung stark sensibilisierter Thrombozyten von der Leber in die Milz verlagern und schließlich auch dort den Abbau verhindern können.

Weiterhin vermögen die Nebennierenrindenhormone die Megakaryozytentätigkeit zu stimulieren. Aufgrund einer Untersuchung an einer 16-jährigen Patientin (Fall 22) konnten wir unter einer Therapie mit 2 mg Prednisolon pro kg Körpergewicht eine Steigerung der Thrombozytenzahlen auf etwa das Zwölffache der Norm bei gleichbleibender Thrombozytenlebensdauer (Abb. 41) beobachten. ASTER und JANDL stellten an einem weiteren Fall eine Steigerung der Produktionsrate der Thrombozyten auf das Achtfache fest (16). Daß neben der Thrombozytenlebensdauer der Knochenmarkfunktion eine Bedeutung für die Thrombozytenzahl im Kreislauf zukommt, ergibt sich auch bei Auftragung der Plättchenertragsflächen und der Thrombozytenzahl in ein Koordinatensystem. Weder in unserem gesamten Material (Abb. 42) noch bei besonderer Berücksichtigung verschiedener unbehandelter und behandelter Thrombozytopenieformen (Abb. 43) fand sich eine strenge Korrelation, wie sie zu erwarten wäre, wenn das Knochenmark keine Regulationsmöglichkeit hätte. Das ist aber von BALDINI behauptet worden, der dem Knochenmark praktisch keine Beeinflussung der Thrombozytenzahl durch entsprechende Vermehrung der Produktion einräumt (18). Zwar sind, wie Untersuchungen nach Plasmapherese ergeben haben, die akuten Reserven des Knochenmarks begrenzt, doch zeigen die nach schweren akuten Blutungen eintretenden, extremen Vermehrungen der Megakaryozytenzahl mit überschießender Thrombozytenproduktion, daß das Knochenmark bedeutsame Fähigkeiten zur Produktionsvermehrung hat.

Aufgrund unserer Untersuchungen lassen sich somit zusammenfassend in Übereinstimmung mit den experimentellen und klinischen Ergebnissen von ASTER und JANDL (16) sowie SHULMAN (179,180) gewisse pathogenetische Mechanismen für die verschiedenen Verlaufsformen der ITP aufzeigen

Bei der akuten Form und den schweren akuten Schuben der chronischen Verlaufsform ist

1 die Thrombozytenlebensdauer auf extrem niedrige Werte (1/2 bis wenige Stunden) verkurzt,
2 der Plattchenertragswert sehr niedrig
3 die Plattchenertragsflache extrem niedrig
4 Über der Leber finden sich hohe initiale Oberflachenaktivitatswerte als Ausdruck eines hepatogenen Abbaus und ein geringer zusatzlicher Aktivitatsanstieg uber der Leber oder Milz

Bei der chronischen Verlaufsform ist dem gegenuber

1 die Thrombozytenlebensdauer auf einige Stunden bis wenige Tage verkurzt
2 der Plattchenertragswert meist, aber nicht immer erniedrigt
3 die Plattchenertragsflache vermindert aber großer als bei akuten Formen
4 Über der Milz finden sich hohe initiale Oberflachen und zusatzliche Aktivitatswerte mit oft weiterem Oberflachenaktivitatsanstieg uber diesem Organ

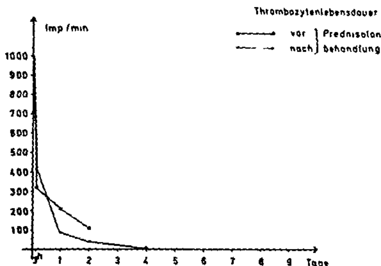


Abb 41 16-jähriges Madchen mit einer chronischen ITP (Fall Nr 22) Thrombozytenlebensdauer vor und nach hochdosierter Prednisolontherapie.
Plattchenzahl vor Behandlung: 9000 / mm³
Plattchenzahl nach Behandlung 111175 / mm³

340 x 10³ Thrombozyten
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20

20 40 60 80 100 120 140 160 180 cm²

Abb 42 Plättchenertragsflächen von 60 Untersuchungen in Beziehung zu den Thrombozytenzahlen.

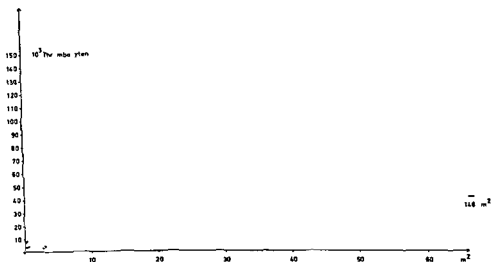


Abb 43 Plättchenertragsflächen und entsprechende Thrombozytenwerte bei 23 Patienten mit behandelten und unbehandelten Thrombopenien unterschiedlicher Aetiologie.

Nr	Name	Alter	Geschl	Bemerkungen	Art der Markierung Autolog A Homolog H	Thrombozyten/ mm^3	Markierungseffekt (%)	Plättchenertr g (%)	Plättchenertrag/ (cm^2)	Thrombozytenlebensdauer (Tg)	Milzaktiv n 24^h (Imp /min)	Leberaktiv n 24^h (Imp /min)	Milz: Leberaktiv Verhältnis	Abbau
Pers nen unter Antikoagulantientherapie (M rcumar)														
35	W P	58	m	Herzinf rkt P I unter 25%	A	288 000	37 9	57 7	117 96	8,3				
36	V P	64	m	Herzinf rkt PI unter 25%	A	343 750	28 o	38 o	93 58	1o 3				
37	W J	78	m	Bein enenthrombose P I unter 25%	A	218 7oo	5o,2	39 4	78 3o	8 1				
38	W B	83	m	Herzinf rkt P I unter 25%	A	2o6 ooo	34 4	48 1	1oo,83	7 6		-		
39	St P	62	m	Her infarkt P I unter 25%	H	35o ooo	37 o	65 8	155 29	8 o				-
Le k amie														
4o	Th M	73	w	akute Leuk mie vor der Untersuch mehrf transf	H	75 ooo		3o,8	32 19	4 6	1133	557	2 o	vorwiegend lienal
41	L S	25	m	chron myel Leukaemie gr Mil tumor polytransfundiert	H	37 46o	4 5	28 o	5 14	2 o	177o	1197	1 5	lienal/ hepatogen
42	E K	61	m	akute Leukaemie	H	45 ooo		55 o	148 83	9 1	164o	89o	1 85	lienal/ hepatogen
Lupus erythematod s														
43	R A	2o	w		H	23 ooo	55 1	28 3	12 5o	2 o	154o	877	1 85	lienal/ hep togen
44	I M	26	w		H	8 ooo	41 8	43 o	1 12	o 3	3231	515	6 3	rein lienal
Hypersplenie syndrom														
45	B W	69	m	Felty Sy drom Milz 6 QF ergröß rt	H	94 ooo	41 9	1o 2	14 o8	8 o	215o	9o8	2 4	vorwiegend lienal
46	B W	48	m	Aetiologie unkl. Milz 4 QF ergrößert	H	39 ooo	31 5	o,	4 3o	8 5	283o	15oo	1 8	li nal/ hepatogen
47	P F	84	m	Aetiologie unbekannt Milz 2 Handbreit ergrößert	H	78 ooo	-	1o 1	16 58	9 1	2893	1181	2 5	vorwiegend lienal
Polyzyth emia ve a														
48	Sch J	64	m	Milz anstoßend	A	46o ooo	38 9	35 7	68 35	7 o	19o4	981	1 9	lienal/ h patogen
Apla i c he Syndrom														
49	H F	7o	w	Milz u Leber nicht ergr	H	61 5oo	25 2	o,o	123 3o	7 4	151o	52o	2 9	vorwiegend lienal
5o	B K	74	m		H	83 4oo		45,o	118 85	6 6	196o	82o	2 35	lie al/ hepatog n

Tab 7 Bestimmung von Lebensdauer und Abbauort bei verschiedenen Erkrankungen.

C. Lupus erythematodes

Der Lupus erythematodes stellt ebenso wie die ITP eine Autoimmunerkrankung dar die oft mit einem Blutplattchenmangel einhergeht. Hinsichtlich Plattchenchenertrag, Plattchenertragsflächen sowie Abbauort bestehen bei dieser Erkrankung im Stadium der Thrombozytopenie deutliche Parallelen zur ITP was beispielhaft in Abb 44 gezeigt ist Sie laßt erkennen wie es entsprechend einem raschen Abfall der Thrombozytenlebensdauerkurve zu einem steilen Anstieg der zusätzlichen Aktivitätswerte über der Milz kommt. Dieser rein lienale Abbau ermoglichte gleichzeitig die Registrierung eines Szintigramms bei dem sich eine normal große Milz darstellte Die ebenso wie hier in einem weiteren Fall (Nr 43) festgestellte Verkurzung der Thrombozytenlebensdauer entspricht den Beobachtungen anderer Autoren (143) Demgegenuber sind Untersuchungen uber den Ort der Thrombozytendestruktion bisher nicht mitgeteilt worden

D Aplastisches Syndrom

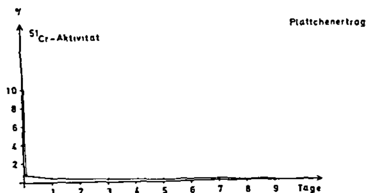
Das aplastische Syndrom unterscheidet sich von den in B und C beschriebenen Immunerkrankungen im Hinblick auf Überlebenszeit und Abbau der Thrombozyten grundsätzlich, was für die Einordnung von unklaren Thrombozytopenien von außerordentlicher Bedeutung ist Wie die Ergebnisse von 2 Patienten (Fall 49 und 50) mit diesem Leiden zeigen (Tab 7 und Abb 45) sind der Plättchenertrag und die Ertragsflächen vollig normal Die Thrombozytenlebensdauer und die Abbauform weichen ebenfalls nicht von der hämatologisch gesunder Patienten ab Die beim aplastischen Syndrom zugrunde liegende primäre Markbildungsstorung ist also durch einen normalen Plättchenertrag eine regelrechte Thrombozytenlebensdauer und einen normalen Abbau in Milz und Leber charakterisiert. Diese Befunde erlauben eine klare Abgrenzung von andersartig bedingten Thrombozytopenien.

E Hypersplenıesyndrom

Mehr oder weniger starke Verminderungen eines oder aller Zellarten des Blutes bei Milzvergrößerungen sind lange Zeit als Ausdruck einer "splenogenen Markhemmung" angesehen worden (52,86a) Die Möglichkeit einer Speicherung von Blutzellen in der Milz ist erstmals von DOAN 1949 diskutiert worden (61) Während für die Erythrozyten neben einer Hamolyse eine vermehrte Ablagerung in der Milz bekannt ist (80) sind die Kenntnisse hier über die Pathogenese der Thrombozytopenie bei Milzvergrößerungen jung und erst in letzter Zeit durch radioaktive Markierung von Thrombozyten und gleichzeitiger *Oberflachenaktivitätsmessungen erweitert* worden (51) Die Verhältnisse konnten bei 3 Patienten (Fall 45,46,47) mit den klinischen Zeichen eines Hypersplenie-syndroms (Milztumor mit Anamie, Thrombozytopenie und/oder Leukopenie) studiert werden

Wie Abb 46 zeigt, sind bereits 30 Minuten nach der Injektion 90% der markierten Plattchen aus der Zirkulation ver*schwunden Dem entspricht der niedrige* Plattchenertrag Bei den 3 Patienten fanden wir Plattchenertragswerte von 0,7 bis 10,2% Der Rest der dann noch zirkulierenden Plättchen lebt normal lang. Diese Tatsache der normalen Überlebenszeit ohne Beachtung der niedrigen Plattchenerträge hat früher Mißverstandnissen Anlaß gegeben Erst die Untersuchung der Oberflachenaktivitaten zeigt, daß wesentliche Unterschiede zu anderen Thrombozytopenieformen vorliegen *In der Zeit*, in der Plättchen aus der Zirkulation verschwinden baut sich über der Milz eine hohe Oberflächenaktivität auf, die dann im wesentlichen konstant bleibt oder - entsprechend dem noch geringen Rest in der Blutbahn befindlicher markierter Plattchen nur gering ansteigt Daraus ergibt sich auch, daß dann ein wesentlicher zusätzlicher Aktivitätsanstieg nicht zustande kommen kann Aus der Tatsache, daß die Überlebenszeit der nach dem initialen Abbau noch verbleibenden Blutplattchen normal ist folgt, daß der Milz beim Hypersplenıesyndrom im Gegensatz zur ITP keine aktiv destruierende Wirkung zukommt, weil sonst ein weiterer Abfall der Blutplättchenaktivität zu erwarten wäre Vielmehr stellt sie einen Speicher für die *Blutplattchen dar* Diese Speicherung macht sich auch in hohen Oberflachenaktivitatswerten uber der Milz bemerkbar, die, wie Abbildung 47 zeigt, im Bereich der bei der ITP ermittelten Werte liegen Man kann der Abbildung weiterhin entnehmen, daß der Aktivitatsanstieg initial ist und im Gegensatz zur chronischen Verlaufsform der ITP dann nicht weiter zunimmt (Abb 29) Daß diese Speicherung reversibel ist und nicht Zelltod bedeutet, ist durch die von ASTER (14) nachgewie*sene Abnahme der Oberflachenaktivität* nach Adrenalingabe, die bekanntlich zu einer Ausschüttung von Blut aus der Milz führt, belegt worden Die Abbildung 48 zeigt die operativ *entfernte Riesenmilz* des in Abb 46 dargestellten Falles Nr 46

Pathophysiologisch kommt demnach die Thrombozytopenie bei Hypersplenie durch eine Speicherung der Blutplattchen in der Milz zustande Das wird auch dadurch bestätigt, daß nach der Splenektomie die Plattchenertragswerte stark ansteigen (55) Diagnostisch laßt sich das Hypersplenie-syndrom über die klinischen Kriterien *hinaus durch einen sehr niedrigen* Plattchenertragswert, eine normale Überlebenszeit der Thrombozyten und hohe Oberflachenaktivitätswerte uber der Milz charakterisieren



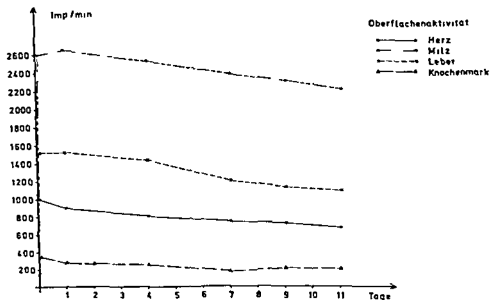


Abb 46 46-jähriger Mann mit einem Hyperspleniesyndrom (Fall Nr. 46).
Der schnelle und hohe Aktivitätsanstieg über der Milz geht mit einem extrem niedrigen Plättchenertrag als Ausdruck einer Speicherung der Thrombozyten in der Milz einher

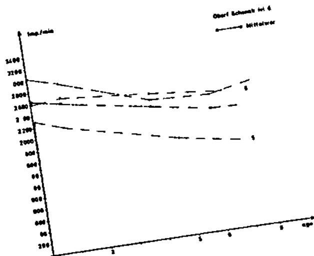


Abb 47 Oberflachenakt vitatswerte über der Milz bei 3 Patienten mit Hypersplenismus.

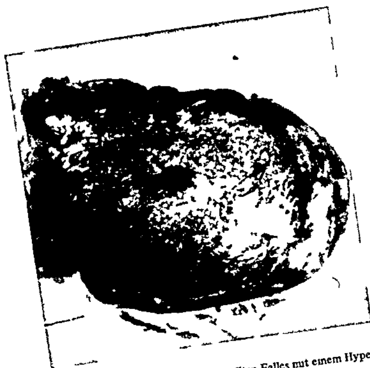
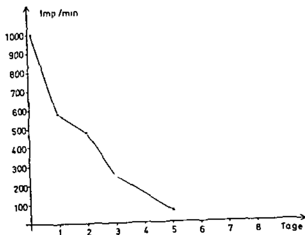


Abb 48 zeigt d e große Milz in Abb 46 dargestellten Falles mit einem Hyperspleniesyndrom (Fall 46) Milzgewicht: 1600 gr



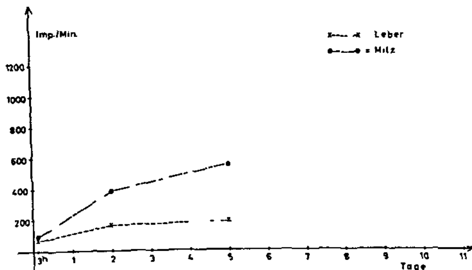


Abb 49 73-jährige Frau mit einer akuten Leukämie (Fall Nr 40)
Die Patientin hatte vor der Untersuchung mehrere Transfusionen erhalten.
Der Abbau der verkürzt lebenden Thrombozyten vollzog sich in Milz und Leber

Leukämien

Bei drei Patienten mit Leukämien wurden unterschiedliche Thrombozytenlebensdauerwerte festgestellt (s Tab 7) Wahrend die *Überlebenszeit der Blutplättchen* bei dem Fall Nr 42 mit einer akuten Leukämie der vor der Untersuchung nie transfundiert worden war normal war und der Abbau sich entsprechend dem von Normalpersonen vollzog lag bei einer 73-jahrigen Frau, die ebenfalls an einer akuten Leukämie erkrankt war, eine Verkurzung der Lebensdauer auf 6 Tage vor (Abb 49) In diesem Zeitraum *stiegen* die zusatzlichen Aktivitatswerte uber der Milz und Leber kontinuierlich an und zeigen damit daß sich der Abbau hier vollzieht Ebenso wie in einem anderen *Fall* (Nr 41) ist die Verkürzung der Thrombozytenlebensdauer wahrscheinlich auf Isoimmunisation *infolge zahlreicher Transfusionen zuruckzu*führen So sind vermutlich auch die von anderen Autoren mitgeteilten unterschiedlichen Überlebenszeiten bei verschiedenen Formen der Leukämie zu bewerten (33 143) NAJEAN (143) fand die Plattchenlebensdauer nur verkürzt wenn die Patienten bereits vorher Bluttransfusionen erhalten hatten

G Weitere Einzeluntersuchungen

Bei einem Patienten mit einer Polyzythämia vera (Fall Nr 48) fanden wir eine normale Überlebenszeit der Thrombozyten die in Milz und Leber abgebaut wurden Je nachdem ob die Thrombozytenzahl erhoht normal oder erniedrigt war sind in der Literatur bei diesem Krankheitsbild verlängerte (12,110) normale oder verkürzte Thrombozytenlebensdauerwerte angegeben worden

Nachdem eine mechanische Zerstorung der Erythrozyten bei angeborenen und erworbenen Herzklappenfehlern und bei Patienten mit künstlichen Herzklappen gesichert ist (79) wurden Untersuchungen bei einem Patienten mit einer mittelschweren Aortenstenose (Fall Nr 52) und einem Patienten mit einer implan-

Nr	Name	Alter J	G hl	B me ku gen	Art der Mar ki *erung* A tolog A Homolog H	Throm bo yt n/ mm³	Markie ungs eff kt (%)	Plätt *hen* ertr g (%)	Plättch n erl ag fl (cm²)	Th *ombozy* te leben d uer (Tg)	Mil akt n 24h (Imp /m i	Leber kt n 24h (Im p /m n)	Mil l l r kt)) lt) s	Abb u
Ve hi d e														
51	G R	44	m	Z td n Impla t ti n einer St rr Edwards Proth se w g n Mitr lin uffizien Polytran f diert	A	228 000	27 3	17 8	26,46	8 3	3897	1087	3 8	rein lie al
52	H G	49	m	Aortenklappe ste o e	A	225 00		41 5	64 35	7 5				
53	H E	54	m	Z t n Sple kton ie wegen portaler Hyperte ion	A	215 000		84 0	172 13	11 8		1030		l p tog n

Tab 8 Lebensdauer und Abbauort bei verschiedenen Einzeluntersuchungen

tierten STARR EDWARDS-Prothese (Fall Nr 51) zur Frage einer Destruktion der Blutplattchen an diesen Ventilen durchgeführt (s.Tab 8) Die Überlebenszeit der Thrombozyten war bei beiden Patienten normal Der rein lienale Abbau bei Fall Nr 51 ist durch eine postoperative Milz vergroßerung zu erklaren LA\DI R hat bei 3 von 7 Patienten mit einer STARR EDWARDS-Prothese eine geringe Verkur zung der Überlebenszeit feststellen können (111)

An zwei Beispielen soll abschließend gezeigt werden, wie die Leber den normalen Abbau nach Splenektomie ubernimmt. In Abb 50 sind die Thrombozytenlebensdauer und die zusatzlichen Aktivitätswerte über der Leber bei einem Patienten mit einem portalen Hochdruck (Fall Nr 53) und bei einer Patientin mit einer ITP Fall Nr 22) nach Milzentfernung gezeigt Man sieht die gute Übereinstimmung der Thrombozytenlebensdauerkurve mit dem Verlauf der zusatzlichen Aktivitätswerte über der Leber Die Splenektomie führt also nicht zu einer Verlängerung der Lebenszeit der Blutplättchen (93) An dem Verlauf der zusätzlichen Aktivitätswerte ist vielmehr zu erkennen daß die Blutplättchen jetzt in der Leber abgebaut werden

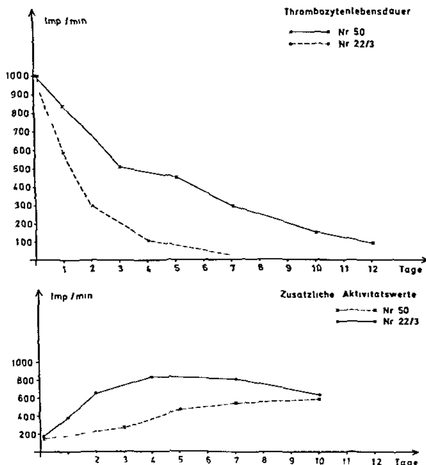


Abb 50 Thrombozytenlebensdauer und zusatzliche Aktivitatswerte uber der Leber bei 2 splenektomierten Patienten. Der Abbau findet entsprechend der Plattchenuber lebenszeit in der Leber statt.

Die Markierung von Thrombozyten in der heute üblichen Methode mit ^{51}Cr gestattet durch Bestimmung der Thrombozytenlebensdauer und semiquantitative Ermittlung des Destruktionsortes eine Einteilung der verschiedenen mit einer Thrombozytopenie einhergehenden Erkrankungen nach pathophysiologischen Gesichtspunkten Wie anhand der im vorhergehenden aufgeführten Beispiele gezeigt wurde, sind in der Pathogenese der Thrombozytopenie 4 Mechanismen von wesentlicher Bedeutung die sich aufgrund der bei der Untersuchung der Plattchenuberlebenszeit mit radioaktiven Substanzen gewonnenen Befunde klar charakterisieren lassen

1 Eine primare Bildungsstorung im Knochenmark

Die Plattchenertragswerte sind normal die Thrombozytenlebensdauer betragt 7 11 Tage Der Abbau findet wie bei Kontrollpersonen in Milz und Leber statt
Die Thrombozytopenie kommt durch eine ungenugende Bildung der verminderten und reifungsgestorten Megakaryozyten im Knochenmark zustande

2 Ein verstarkter peripherer Abbau

Die Plattchenertragswerte sind in Abhangigkeit von der Antikorperaktivitat fast regelmaßig vermindert besonders bei akuten Verlaufsformen der ITP Die Thrombozytenlebensdauer ist immer mehr oder weniger stark verkurzt Der Abbau der Blutplattchen findet verstarkt in dem RES von Milz und Leber statt Unsere bisher vorliegenden Befunde zeigen in Übereinstimmung mit den wenigen Mitteilungen in der Literatur (16,179), daß der Abbau bei den besonders foudroyant und mit extrem verkurzter Lebensdauer verlaufen den akuten Formen und schweren Schuben der ITP ganz vorwiegend im RES der Leber stattfindet (hepatogener Abbau) wahrend bei den chronischen Verlaufsformen mit weniger starker Verkurzung der Lebensdauer die Milz Ort der verstarkten Destruktion ist

3 Primare Bildungsstorung und verstarkter peripherer Abbau

Hier finden sich die unter 2 genannten Kriterien Zusatzlich lassen sich Verminderungen und Reifungsstorungen im Knochenmark nachweisen

4 Speicherung der Thrombozyten in einer vergroßerten Milz (sog. Hypersplenie syndrom)

Extrem niedrige Plattchenertrage und Plattchenertragsflachenwerte, normale Lebensdauer der Thrombozyten und hoher, bereits initialer Oberflachenaktivitatswert uber der Milz ohne weiteren Aktivitatsanstieg sind die Charakteristika dieser Form Die Aufklarung dieser Form ist erst in jungster Zeit (14 78) moglich geworden

1 Aus der obigen Zusammenstellung ergibt sich daß bei Auswertung aller bei Markierung von Blutplattchen mit ^{51}Cr gewonnenen Befunde, also im einzelnen der Überlebenszeit der Thrombozyten des Plättchenertrages, der Plättchenertragsflächen und der Oberflachenaktivitaten eine Aufklarung der Pathogenese einer Thrombozytopenie und damit unter Umstanden auch eine ätiologische Einordnung moglich ist.

2 Die Methode erlaubt weiterhin durch die nach Übertragung von Plasma mit thrombozytaren Isoantikorpern und solchen von Patienten mit einer ITP erhobenen Befunde Aufschlusse über Verbleib und Destruktion zu erlangen Die besonders in den letzten 2 Jahren gewonnenen Erkenntnisse uber die Pathogenese der idiopathischen Thrombozytopenie werden auf diese Weise noch sicher erweitert werden Die Erfolgsaussichten einer Thrombozyten transfusion lassen sich sicher beurteilen So resultiert beispielsweise aus dem auf diese Weise festgestellten bei der akuten Verlaufsform der ITP vorliegenden rapiden Plattchenzerfall die Nutzlosigkeit einer Thrombozytentransfusion (173)

3 Die Lebensfähigkeit der unter den verschiedenen Bedingungen konservierten Thrombozyten und solchen von blutgruppengleichen Spendern (15 160) laßt sich verlaßlich nur durch Verfolgung der an die Thrombozyten gebundenen Radioaktivitat beurteilen (173) Fur Studien zur Thrombozytenkonservierung ergibt sich daraus die Notwendigkeit einer radioaktiven Markierung.

4 In Analogie zu den Verhältnissen bei hamolytischen Anämien ist man geneigt aus dem beim lienalen Abbautyp vorhandenen hohen Radioaktivitatsanstieg uber der Milz die Erfolgsaussicht für eine Splenektomie herzuleiten Diese Überlegung ist umso verlockender als es für die Indikation zur Splenektomie bei der chronischen ITP bislang kein verlaßliches Kriterium gibt (32) Unsere eigenen Ergebnisse lassen hier eine gesicherte Aussage noch nicht zu

Bei 3 Patienten mit einem lienalen Abbautyp wurde eine Splenektomie durchgeführt Sie war einmal erfolgreich (Fall Nr 32) einmal führten minimale Prednisolondosen zu einer Normalisierung der Plattchenzahlen (Fall Nr 22) Bei einem weiteren Patienten kam es zwar nur zu einem ganz geringen Anstieg der Thrombozytenzahl von 5 000 auf 45 000 pro mm^3 (Fall Nr 30) Die vorher starke hamorrhagische Diathese verschwand aber vollig Die Ursache dieser inkompletten Remission ist uns bisher nicht bekannt Moglich ist daß das RES der Leber den Abbau jetzt ubernommen hat Eine Kontrolluntersuchung konnte leider nicht durchgeführt werden Bei 2 weiteren Patienten mit einem Hyperspleniesyndrom (Fall 45 und 46 und Abb 48) war die Splenektomie erfolgreich.

Die im ubrigen bisher mitgeteilten Ergebnisse sind insgesamt sehr ermutigend NAJEAN und Mitarbeiter (141) die 41 Patienten 1 Jahr nach Milzentfernung untersuchten stellten eine Korrelation zwischen dem Erfolg der Splenektomie und lienalem Abbautyp fest Ähnliche Befunde liegen in der Literatur sonst nur von CASTALDI und FIRKIN (40) vor

ZUSAMMENFASSUNG

Die Untersuchung von Lebensdauer und Abbauort menschlicher Thrombozyten gibt Antwort auf die Frage nach dem Verbleib und Verhalten der Blutplättchen im Kreislauf nach ihrer Bildung im Knochenmark Fur diese Bestimmungen sind verschiedenartige Methoden entwickelt worden, nachdem Anfang dieses Jahrhunderts erstmals anlaßlich von Blutubertragungen festgestellt worden war, daß die Thrombozyten im Empfangerkreislauf etwa 3 - 4 Tage leben Aus einer zusammenfassenden Übersicht uber die bisher angewendeten Methoden zur Bestimmung der Thrombozytenlebensdauer geht hervor daß die Verwendung radioaktiver Isotope ganz neue Einblicke in die Thrombozytenphysiologie ermoglicht hat Von allen im einzelnen aufgeführten Isotopen kommen für die Routineanwendung in der Klinik nur die Markierung der Blutplattchen mit P^{32}-Diisopropylfluorophosphat (DFP^{32}) und ^{51}Cr infrage Gegenuber DFP^{32} hat ^{51}Cr den entscheidenden Vorteil, daß gleichzeitig mit der Thrombozytenlebensdauerbestimmung durch Messung der Oberflachenaktivitat an verschiedenen Stellen des Körpers der Abbauort der Thrombozyten sicher ermittelt werden kann So ist die Markierung der Blutplattchen mit Natriumchromat zur heute gebrauchlichsten Methode geworden

In Abwandlung der Methode von AAS und GARDNER wurde eine Technik entwickelt mit der große Chrommengen an die Thrombozyten gebunden werden konnen Es ergab sich dabei daß der Markierungseffekt mit der Thrombozytenzahl, der absoluten Menge inkorporierter Radioaktivitat und der Konzentration der Blutplättchen zunimmt In folge der Verwendung hoher spezifischer Aktivitaten von 78-347 mC/mg Cr kommt es nicht zu einer toxischen Schädigung durch Natriumchromat Die Aufarbeitung in vitro hat allerdings zur Folge daß etwa 40-45% der inkubierten Blutplattchen nach Injektion nicht mehr lebensfahig sind Da eine Elution und Reutilisation von Natriumchromat nicht eintritt, ist dieses Isotop als optimale Markierungssubstanz anzusehen

Unsere Studien erstreckten sich auf 62 Einzeluntersuchungen bei 53 Patienten, und zwar 21 Kontrollpersonen, 13 Patienten mit einer idiopathischen Thrombozytopenie, 5 Personen wahrend Antikoagulantientherapie 3 Patienten mit einer Leukamie 2 Falle von Lupus erythematodes 3 Patienten mit einem Hyperspleniesyndrom 2 Patienten mit einem aplastischen Syndrom, 1 Polyzythamia vera je einen Patienten mit einer Aortenklappenstenose einer STARR EDWARDS-Prothese und mit Zustand nach Splenektomie

Bei Normalpersonen findet man durch autologe Ermittlung der Thrombozytenlebensdauer nach Korrektur für den täglichen Radioaktivitatsverlust eine bis zum Wert von 10 % der Ausgangsaktivitat praktisch linear verlaufende Kurve, die von diesem Punkt aus in einen exponentiellen Teil ubergeht Der lineare Verlauf weist daraufhin, daß der Abbau der Thrombozyten im Normalfalle sich als reiner Alterungsvorgang vollzieht. Unterschiede hinsichtlich der Kurvenform und der Zeit bestehen weder bei autologer noch bei homologer Transfusion Da der Kurvenverlauf bis etwa zum 10 %-Wert der Ausgangsaktivitat annähernd linear ist und der dann folgende exponentielle Anteil unterschiedlich steil abfallt, haben wir als Maß für die Thrombozytenlebensdauer den 10 %-Wert der Ausgangsaktivitat zugrunde gelegt. Bei Normalpersonen betrug die Thrombozytenlebensdauer 8 3 (7 2 9,9) Tage mit einem taglichen Abfall von 12%

Fur die Bewertung von pathologischen Zustanden sind neben der Thrombozytenlebensdauer und den Oberflachenaktivitätswerten der Plättchenertrag und die Plattchenertragsflache bedeutungsvoll Der Anteil der in der Peripherie zirkulierenden, lebensfähigen markierten Thrombozyten ist der Plättchenertrag Bei Normalpersonen betragt er in der angegebenen Technik durchschnittlich 55%

Zur Beurteilung des Thrombozytenabbaues sind sowohl die absoluten Oberflächenaktivitatswerte als auch die sogenannten zusatzlichen Aktivitatswerte heranzuziehen Darunter versteht man bei Zugrundelegung der in thrombolytischer Hinsicht inaktiven Herzaktivitat nach dem Vorschlag von HUGHES-JONES und SZUR den Unterschied zwischen theoretisch zu erwartendem und tatsächlich eingetretenem Aktivitatsanstieg oder abfall uber verschiedenen Organen Die Messung unmittelbar nach der Injektion der markierten Thrombozyten mit entsprechender Berechnung der zusätzlichenAktivitatswerte hat sich deshalb als zweckmaßig erwiesen weil eine bei einer Reihe pathologischer Zustande vorhandene rasche Zerstorung der Blutplättchen nur so erfaßt werden kann Im Normalfalle steigen die zusatzlichen Aktivitatswerte innerhalb der ersten 12-17 Minuten uber der Milz auf maximal 1000 Impulse pro Minute und uber der Leber auf hochstens 100 pro Minute an Dieser fruhe Aktivitatsanstieg ist Ausdruck der Ablagerung der bei der Aufarbeitung geschädigten Thrombozyten woraus hervorgeht, daß die durch die Markierung ladierten Blutplattchen vor allem in der Milz und daneben in geringerem Umfang in der Leber zerstort werden Jeder zeitlich und großenmaßig daruberhinaus gehende Aktivitatsanstieg in den ersten Stunden ist als vermehrte Thrombozytendestruktion in dem betreffenden Organ anzusehen Bei einem sehr raschen Plattchenabbau kann ein zusatzlicher Aktivitatsanstieg nicht mehr eintreten Dann mussen die absoluten Oberflachenaktivitatswerte für die Beurteilung des Abbauortes berucksichtigt werden. Hierfür haben wir als vergleichbare Große das Milz zu Leberaktivitatsverhaltnis nach 24 Stunden zugrunde gelegt. Bei Normalpersonen ist dieses Verhaltnis 1 5 zu 1 Bei Berucksichtigung von Vergleichsuntersuchungen am Phantom ergab sich aus diesem Verhaltnis, daß der Abbau der Thrombozyten bei Normalpersonen zu gleichen Teilen in der Leber und in der Milz vor sich geht. Nimmt man dieses Verhältnis als Maßstab so laßt sich semiquantitativ bestimmen, ob der Abbau lienal-hepatogen, vorwiegend bzw rein lienal oder vorwiegend bzw rein hepatogen erfolgt.

Fur die Diagnostik der idiopathischen Thrombozytopenie (ITP) ist die Bestimmung der Überlebenszeit der Blutplattchen das verlaßlichste Kriterium In jedem Stadium der Erkrankung wird die Thrombozytenlebensdauer offenbar in Abhängigkeit von der Antikorperaktivität auf Werte von wenigen Stunden bis einigen Tagen verkürzt gefunden. Dabei sind die stärksten Verminderungen bei den hochakuten Verlaufsformen und dem akuten Schub der chronischen ITP festzustellen wahrend sie im Intervall bei den chronischen Verlaufsformen zwar meist aber nicht immer reduziert ist.

Fur die idiopathische Thrombozytopenie konnten verschiedene Abbautypen herausgearbeitet werden Grundsätzlich kann die vermehrte Destruktion in der Milz (rein oder vorwiegend lienal) in der Leber (hepatogen) oder in Milz und Leber (kombiniert lienal hepatogen) stattfinden Charakteristisch für den lienalen Abbau sind ein Milz zu-Leber Aktivitätsverhaltnis nach 24 Stunden uber 2 0 1 hohe Oberflachenaktivitätswerte über der Milz sowie ein starker die erste 1/4 Stunde überdauernder zusatzlicher Aktivitatsanstieg über diesem Organ der sich spiegelbildlich zu dem Abfall der Thrombozytenlebensdauerkurve verhalt.

Beim hepatogenen Abbau liegen dagegen die Kennzeichen einer extrem raschen Thrombozytenstorung vor Plättchenerträge von meist weniger als 10% der Ausgangsaktivität und Überlebenszeiten von einer halben bis wenigen Stunden gehen mit initial hohen Oberflachenaktivitatswerten uber der Leber einher denen ein nur geringer weiterer zusätzlicher Aktivitatsanstieg uber Leber und Milz folgt Bei 12 Patienten mit einer chronischen idiopathischen Thrombozytopenie war die

Milz 8 mal allein oder vorwiegender Abbauort 3 mal *wurden die Thrombozyten in der* Leber zerstort und einmal erfolgte die Destruktion kombiniert lienal-hepatogen Die starke Aktivitatsansammlung in der Milz beim lienalen Abbautyp konnte erstmals szintigraphisch objektiviert werden

Im Hinblick auf die Zuordnung der Verlaufsformen der idiopathischen Thrombozytopenie zu den bei der Markierung von Blutplattchen mit ^{51}Cr gewonnenen Befunden ließ sich nachweisen daß der Abbau der Thrombozyten *bei der akuten Verlaufsform* und im akuten Schub in der Leber vor sich geht, wahrend bei den chronischen Formen die Milz Hauptabbauort ist Bei maßig schweren Schuben der ITP konnen dagegen Übergange in Form eines kombiniert lienal-hepa*togenen Abbaues beobachtet werden*

Die Messung der Plasma und Urinradioaktivitat sowie die Bestimmung der Menge des Isotops *in operativ entfernten Milzen* haben gezeigt, daß die Thrombozyten weder im Normalfalle noch bei vermehrter Destruktion intravasal zerstort werden. Vielmehr vollzieht sich der Abbau organgebunden im reticuloendothelialen *System* von Milz und Leber

Die Veranderungen, die sich spontan, wahrend der Behandlung mit Kortikoiden oder nach Splenektomie bei der idiopathischen Thrombozytopenie abspielen, *sind* sicher mit Hilfe der radioaktiven Markierung von Thrombozyten zu erkennen Die bisher mitgeteilten Verlaufsbeobachtungen beschranken sich auf ganz vereinzelte Fälle Die eigenen Untersuchungen lassen erkennen daß bei der ITP *mit dem* Übergang *vom akuten* Stadium in die Remission eine Verlagerung des Abbauortes von der Leber zur Milz stattfindet. Dasselbe ist der Fall wenn Kortikoide zur Anwendung gelangen Die Nebennierenrindenhormone entfalten *ihre* Wirkung *nicht* uber eine Verlangerung der Thrombozytenlebensda er sondern sie führen zu einer Verlagerung d s Abbauortes. Das im akuten Stadium besond aktive RES der Leber erkenntlich a der ar rfolgenden hepatogenen Abbau wir h di *Kortikoide blockiert und* die Dest ion der Thrombozyten in die Milz verlagert Schließlich vermogen die Nebennierenrindenhormone *auch den Abbau der* Blutplattchen in der Milz zu verhindern Aus dem Vergleich der Thrombozytenzahl und der Plättchenertragsflache sowie dem Anstieg der Thrombozytenzahlen bei unveranderter Überlebenszeit geht hervor, daß die Kortikoide *daruberhinaus einen stimulierenden* Effekt auf die Thrombopoese ausuben

Aus unseren Ergebnissen und Verlaufsbeobachtungen von Patienten mit idiopathischer *Thrombozytopenie leiten sich im Zusammen*hang mit den experimentellen Untersuchungen von ASTER und SHULMAN Gedanken zur Immunpathogenese der ITP her Der plattchenzerstorende Faktor bei der ITP ist *eine spezies-spezifisch wirksame Substanz* die *von Plättchen absorbiert wird sich in der 7 S-*Gamma-Globulinfraktion des Plasmas befindet und in vivo einen den Isoimmunantikorpern quantitativ und qualitativ gleichwertigen Effekt hat. Der Faktor besitzt dementsprechend Antikorpercharakteristik. Das Vorhandensein dieses sogenannten ITP Faktors hat zur Folge daß eigene und transfundierte Thrombozyten verkurzt leben

Die dadurch bedingte vermehrte Destruktion *der Thrombozyten vollzieht sich in* Abhangigkeit von dem Grad ihrer Sensibilisierung bzw der Potenz der Antikorper organgebunden in verschiedenen Anteilen des RES Bei den chronischen Verlaufsformen ist die Milz *Hauptabbauort* wahrend bei den akuten Ver*laufsformen* und *im akuten Schub der ITP* die Leber die Grabstatte der Thrombozyten darstellt Das erklart die dem Chirurgen seit langem bekannte hohe Operationsmortalitat bei der akuten ITP Der Erfolg einer Milz*entfernung bei der idiopathischen Thrombo*zytopenie ist auf die Beseitigung des Hauptabbauortes zuruckzuführen Andererseits hat die Splenektomie keine Verlangerung der Thrombozytenlebenszeit zur Folge Vielmehr *werden die Blutplattchen dann in der Leber* zerstort Beim floriden Lupus erythematodes liegen ahnliche Verhältnisse vor wie bei der idiopathischen Thrombozytopenie Auch hier finden sich Überlebenszeiten von wenigen Stunden *bis einigen Tagen* Bei unseren Patienten bestand ein lienaler Abbautyp

Bei den primären Bildungsstörungen, wie sie beim aplastischen Syndrom vorhanden sind, werden dagegen Verhältnisse wie bei Normalpersonen beobachtet. Im einzelnen ... die übertragenen Thrombozyten 7 11 ... die Plättchenerträge und Plattchenerträgsflächen sind normal. Der Abbau der Thrombozyten vollzieht sich wie bei hämatologisch gesunden Personen zu gleichen Teilen in Leber und Milz.

Die radioaktive Markierung von Blutbestandteilen hat die Pathogenese der Thrombozytopenie beim Hyperspleniesyndrom in ein neues Licht gerückt. Die Theorie einer ...nogenen Markhemmung ist dadurch hinfällig geworden. Vielmehr stellt die Milz beim Hyperspleniesyndrom einen großen Speicher dar, in dem sich die Thrombozyten sammeln und lebensfähig verbleiben. Das findet seinen Ausdruck in gegenüber Normalpersonen sehr niedrigen Plättchenertragswerten von 10% der Ausgangsaktivität und weniger, einer normalen Überlebenszeit der restlichen zirkulierenden Thrombozyten und hohen initialen Oberflächenaktivitatswerten über der Milz.

Die Milz spielt also bei den ätiologisch verschiedenen Thrombozytopenieformen eine unterschiedliche Rolle. Wahrend bei der ITP und dem Lupus erythematodes das lienale RES aktiv an der Destruktion der Thrombozyten beteiligt ist, kommt die Thrombozytopenie beimHyperspleniesyndrom durch eine Speicherung der Blutplättchen in der Milz zustande.

Nach den durch Bestimmung der Thrombozytenlebensdauer und semiquantitative Bestimmung des Abbauortes gewonnenen Erkenntnisse sind 4 Mechanismen in der Pathogenese von Thrombozytopenien bedeutungsvoll:

1. Eine primare Insuffizienz des Knochenmarks, wodurch es zu einer ungenugenden Bildung der Thrombozyten kommt.
2. Eine vermehrte Zerstorung der Blutplattchen in der Peripherie. Dabei ist die Thrombozytenlebensdauer immer mehr oder weniger stark verkurzt und das RES von Milz und Leber übernehmen den verstärkten Abbau der Blutplättchen.
3. Kombinierte Bildungsstörung und verstärkter Abbau in der Peripherie.
4. Speicherung der Thrombozyten beim sogenannten Hyperspleniesyndrom.
   Als charkteristisch hierbei sind extrem niedrige Plättchenertrage und Plattchenertragsflachen sowie initiale hohe Oberflächenaktivitätswerte über der Milz ohne weiteren Aktivitatsanstieg anzusehen.

Welche Moglichkeiten bietet die Anwendung der radioaktiven Markierung von Thrombozyten mit ^{51}Cr?

1. Überlebenszeit der Thrombozyten, Plättchenertrag und Plättchenertragsflächen sowie Oberflachenaktivitätswerte sind Daten, die unter Berücksichtigung der klinisch-hamatologischen Befunde eine Aufklärung der Pathogenese einer Thrombozytopenie und ihre atiologische Einordnung ermöglichen.
2. Die Lebensfähigkeit von Thrombozyten wird zur Zeit durch keine andere Große besser reprasentiert als durch die Thrombozytenlebensdauer. Für Konservierungsstudien von Thrombozyten ist daher eine radioaktive Markierung erforderlich.
3. Verlaufsbeobachtungen bei verschiedenen Erkrankungen sowie experimentelle Studien mit antikorperhaltigen Plasmen erlauben durch Verfolgung der markierten Thrombozyten im Kreislauf Einblick in den speziellen Mechanismus verschiedener Thrombozytopenien. Dadurch ist eine weitere Aufklärung der in den letzten Jahren gewonnenen Erkenntnisse sicher zu erwarten.

SUMMARY

The examination of the lifespan and site of destruction of human thrombocytes answers the question as to the distribution and the behaviour of blood platelets in the circulation after their formation in bone marrow From a comprehensive review of the methods that have been used up till now to determine the lifespan of thrombocytes, it can be seen that the use of radioactive isotopes has opened up completely new aspects of thrombocyte physiology From all the isotopes itemised only the labelling of the blood platelets with P^{32}-diisopropylfluorophosphate (DFP^{32}) and sodiumchromate ($Na_2{}^{51}CrO_4$) can be considered further for routine clinical use Compared with DFP^{32} ^{51}Cr has the decisive advantage that the site of destruction of the thrombocytes can be ascertained by measurement of the surface activity at various regions of the body simultaneously with the determination of their lifespan The labelling of blood platelets with radiochromate has therefore today become the most usual method

In modification of the method of AAS and GARDNER a technique was evolved by which large quantities of chromium could be bound to the thrombocytes The results showed that the labelling effect increases with the number of thrombocytes the absolute quantity of incorporated radioactivity and the concentration of blood platelets As a consequence of the use of high specific activities of 78 347 mC/mg, toxic damage by radiochromate does not occur Nevertheless, the drawback of the labelling procedure in vitro is that some 40 45% of the incubated blood platelets are not longer viable after injection As an elution and reutilisation of natrium chromate does not take place this isotope may be regarded as the optimal labelling substance

Our studies extended to 62 individual investigations with 53 patients twenty one control persons, thirteen patients with an idiopathic thrombocytopenia five persons during anti-coagulant therapy, three patients with a leucemia two cases of lupus erythematodes three patients with a hypersplenism, two patients with an aplastic syndrome, one polycythaemia vera, one patient with an aortic valve stenosis one with a STARR EDWARDS prosthesis and one after splenectomy

In normal persons by autologous tagging after correction for the daily loss in radioactivity a curve is recorded which declines practically linear up to the value of 10% of the initial activity ending in an exponential part The linear course shows that the destruction of the thrombocytes in normals takes place as a pure process of ageing Differences regarding the shape of the curve and the lifespan do not exist either with autologous or homologous transfusions As the shape of the curve up to about the 10% value of the initial activity is almost linear and the following exponential part falls with varying steepness, we measure the lifespan of thrombocytes according to the 10% value of the initial activity In normal persons the thrombocyte lifespan was 8 3 days (7 2 9 9), with a daily drop of about 12%

For the evaluation of pathological conditions in addition to the platelet lifespan and the surface activity values, the platelet yield and the platelet yield area are important The percentage of the viable labelled thrombocytes in the circulation is the platelet yield In normal persons it averages 55% in our technique

For the assessment of the destruction of the thrombocytes the absolute surface activity values as well as the additional activity values have to be taken into account Taking the surface activity over the thrombolytic inactive heart the additional activity values are calculated from the difference between the theoretically to be expected and the actually measured increase or decrease of activity

Measuring the labelled thrombocytes immediately after injection with corresponding calculation of the additional activity values has therefore proved practicable since rapid destruction of the platelets occurring in a number of pathological conditions can only in this way be comprehended In normal cases the additional activity values increase within the first 12 to 17 minutes over the spleen to a maximum of 1 000 counts/min and over the liver to a maximum of 100 counts/min This early increase in activity is an expression of the deposition of the thrombocytes damaged by the tagging procedure Apparently the platelets injured by the labelling are destroyed above all in the spleen and to a lesser extent in the liver Every increase in activity in the first hours beyond this in time and degree may be regarded as growing platelet destruction in the respective organ In cases of a very fast platelet destruction an additional increase in surface activity can no longer take place Then the absolute surface activity values for the assessment of the destruction site must be considered. For comparison we have taken as a basis the ratio of spleen to liver activity after 24 hours. In normal persons it is 1 5 By comparing experiments with a phantom it was shown from this ratio that the destruction of thrombocytes normally takes place to the same degree in the liver and in the spleen. From the spleen to liver activity ratio it can be determined semi-quantitatively whether the destruction occurs in spleen and liver mainly or solely in the spleen or mainly or solely in the liver

For the diagnosis of the idiopathic thrombocytopenia (ITP) the determination of the survival time of the blood platelets is the most reliable criterion In every stage of the disease the platelet lifespan is reduced to values from a few hours to some days The greatest diminution is to be found in the acute forms and the acute relapses of the chronic ITP During remissions of the chronic type platelet survival is usually but not always reduced.

Various destruction types of the idiopathic thrombocytopenia could be detected. Principally, the increased destruction can take place solely or mainly in the spleen or in the liver or in spleen and liver Typical for the splenic destruction is a spleen liver activity ratio after 24 hours of 2 0 1 high surface activity values over the spleen as well as a considerable additional rise in activity lasting longer than the first 15 mts. over this organ and this increase corresponds to the fall in the platelet survival curve

The signs of an extremely fast platelet destruction are evident in hepatic destruction platelet yields of generally less than 10% of the initial activity with survival times of half an hour to some few hours accompany early high surface activity values over the liver followed by a merely small further additional activity rise over liver and spleen. Of twelve patients with a chronic idiopathic thrombocytopenia the spleen in eight was the sole or principal destruction site in three the thrombocytes were destroyed in the liver, and once combined destruction in spleen and liver was found.

It could be proved that in the acute form and in the acute relapse of ITP the thrombocytes are destroyed mainly in the liver During remissions the spleen is the principal site of destruction. With moderately severe relapses of the ITP on the other hand transitions in the form of a combined destruction in both liver and spleen can be observed.

The measurement of the plasma and urine radioactivity as well as the determination of the quantity of the isotopes in spleens removed by operation have shown that the thrombocytes are not destroyed intravasculary either in the normal case or in increased destruction Rather are the platelets segregated in the reticuloendothelial system of spleen and liver

The changes which spontaneously take place during treatment with corticoids or after splenectomy in the course of the idiopathic thrombocytopenia are definitely to be recognised by means of the radioactive platelet labelling. The hitherto published reports are limited to purely individual cases According

to our own results in patients with ITP a shift of the site of destruction takes place from the liver to the spleen when the acute stage changes into a remission This is true also when corticoids are used. These hormones do not develop their effect by prolonging the lifespan but lead to a shift of the destruction site The reticuloendothelial system of the liver especially active in acute phases is blocked by the corticoids and the destruction of the thrombocytes is shifted into the spleen. Finally the corticoids are also capable of preventing the destruction of the blood platelets in the spleen From the comparison of the thrombocyte number and the platelet yield areas as well as the increase in thrombocyte numbers with unchanged survival time, it is apparent that the corticoids also stimulate the thrombopoesis.

From our results and course observations of patients with idiopathic thrombocytopenia in association with the experimental investigations of ASTER and SHULMAN, some conclusions on the pathogenesis of the ITP are drawn. The antiplatelet factor in the ITP is a species-specific effective substance which is absorbed by the platelets, can be found in the 7 -S-Gammaglobuline fraction of the plasma, and has in vivo quantitative and qualitative equivalent effect to isoimmunantibodies. The factor therefore has antibody characteristics. As a consequence of this ITP factor, survival time of the patient s own and transfused thrombocytes is reduced. The increased destruction of the thrombocytes in ITP takes place in dependence on the degree of the platelet antibody-titer in various parts of the reticuloendothelial system. In the chronic form the spleen is the main destruction site, while in the acute form and in the acute relapse of the ITP the liver represents the grave of the thrombocytes. The success of the removal of a spleen in idiopathic thrombocytopenia is to be traced back to the removal of the main destruction site On the other hand, the splenectomy does not result in the lengthening of the platelet lifespan. Rather are the blood platelets then destroyed in the liver In acute lupus erythematodes similar conditions are present as in idiopathic thrombocytopenia here too there are survival times of the thrombocytes ranging from a few hours to some days In our patients a splenic destruction type was found.

In primary bone marrow disease with reduced platelet production as for instance in the aplastic syndrome, on the contrary conditions as in normals were observed the transfused thrombocytes live for 7-11 days, the platelet yields and platelet yield areas are normal The destruction of the thrombocytes is accomplished as in haematologically healthy persons equally in liver and spleen

The radioactive labelling of blood platelets has brought new aspects to the pathogenesis of the thrombocytopenia in hypersplenism The theory of a blockade of the bone marrow by the enlarged spleen has become untenable On the contrary in hypersplenism the spleen represents a large pool in which the thrombocytes are collected and remain viable Very low platelet yields of 10% and less of the initial activity, a normal survival time of the remaining circulating thrombocytes and high initial surface activity over the spleen are typical findings.

Thus in the etiologically different thrombocytopenias the spleen plays a variable role while in the ITP and the lupus erythematodes the splenic reticuloendothelial system is actively engaged in the destruction of the thrombocytes the thrombocytopenia in hypersplenism is caused by a storage of the platelets in the spleen.

According to the results arrived at by the determination of the thrombocyte lifespan and semi-quantitative estimation of the site of destruction, four mechanisms in the pathogenesis of thrombocytopenias are important.

1 A primary insufficiency of the bone marrow, leading to an insufficient production of the thrombocytes.
2. An increased destruction of the blood platelets in the periphery thereby the thrombocyte lifespan is always more or less severely shortened. The reticuloendo-

thelial system of spleen and liver take ve the heightened destruction

3 Combined production disturba... increased destruction in the perip...

4 Pooling of the thrombocytes in splenism Characteristic for this s... are extremely low platelet yi... platelet yield areas as well as ... surface activity values over the ... without further increase of activi...

Herrn Professor Dr. F. Grosse-Brockhoff (Direktor der I. Medizinischen Klinik der Universität Düsseldorf) bin ich für die Überlassung von Krankengeschichten und Befunden, die ich während meiner Tätigkeit an seiner Klinik bearbeiten konnte, zu großem Dank verpflichtet.

Herrn Professor Dr. G. Gehrmann (Chefarzt der Medizinischen Klinik der Städtischen Krankenanstalten Wuppertal-Barmen) danke ich für Hinweise und Ratschläge.

1 AAS K A
Labelling of blood platelets with ^{51}Cr
Proc. VIIeme Congr Europ Soc Haemat.
London 1959

2 AAS K A. a. F H. GARDNER
Survival of blood platelets with chromium 51
J Clin. Invest. 37 1257 (1958)

3 AAS K A a. F H. GARDNER
Live span of normal human platelets tagged with chromium 51
Proc. sixth Congr int Soc. Blood Transf
New York 1956

4 ACKROYD J F
Pathogenesis of thrombocytopenic purpura due to hypersensitivity to sedormid (allyl-isopropyl acetylcarbamide)
Clin. Sci. 7 249 (1949)

4a ADAM W HEIMPEL, H. GUMP K D HOFFMANN G u. KEIDERLING W
Die Bestimmung der Thrombozytenlebenszeit mit Diisopropylfluorophosphat (DFP32)
Blut 14, 155 (1966)

5 ADELSON E
Platelet tagging with tritium labelled diisopropylfluorophosphate
Blood 26 744 (1965)

6 ADELSON E. R. M. KAUFMANN A.A LEAR, J C. KIRBY a. J.J RHEINGOLD
Physiology of platelet destruction as revealed by tagging of cohorts.
I studies in dogs.
J Lab & Clin Med 62 385 (1963)

7 ADELSON E. R M KAUFMANN A A LEAR a. J J RHEINGOLD
Platelet tagging with tritium labelled diisopropylfluorophosphate
Congr Int Soc. Haemat. Stockholm 1964
Abstract Nr 2

8 ADELSON E J J RHEINGOLD and W H. CROSBY
Studies of platelet survival by tagging in vivo with P32
J Lab & Clin Med 50 570 (1957)

9 ADELSON E. J J RHEINGOLD A A. LEAR, J ISBISTER J C. KIRBY and R. M KAUFMANN
Continuous in vivo coagulation, the determinant of platelet survival.
Blood 18 796 (1961)

10 ADELSON E J J RHEINGOLD O PARKER, A. BUENAVENTURA a. W H. CROSBY
Platelet and fibrinogen survival in normal and abnormal states of coagulation.
Blood 17 267 (1961)

11 ADELSON E a. M. STEFANINI
Studies of platelets.
VI Demonstration and characterisation on a heterologous (FORSSMANN) platelet agglutinin
Blood 7 700 (1952)

12 ALFOS L. G E. O FIELD a. E.M LEDLIE
Clinical studies with DFP32 on the lifespan of platelets.
Lancet 2 941 (1959)

13 ASHBY E.
The determination of the length of life of transfused blood corpuscels in man
J exp Med. 29 167 (1919)

14 ASTER R H.
Pooling of platelets in the spleen. role in the pathogenesis of the "hypersplenic" thrombocytopenia.
J Clin. Invest 45 645 (1966)

15 ASTER, R. H.
Effect of an anticoagulant and ABO-incompatibility on recovery of transfused human platelets.
Blood 26 732 (1965)

16 ASTER R H. a. J H. JANDL
Platelet sequestration in man.
J Clin. Invest. 43 843 (1964)
43 869 (1964)

17 ATHENS R.J W., A M. MAURER H. AESENBRUCKER G E.CARTWRIGHT a. M M WINTROBE
Leucocinetic studies I A method for labelling leucocytes with DFP32
Blood 14 303 (1959)

18 BALDINI M
Idiopathic thrombocytopenic purpura.
New Eng. J Med 274 1245 (1966)
274 1301 (1966)
274 1360 (1966)

19 BALDINI M.
Platelet survival
Proc. 10th Congr Europ Soc. Haemat.
Straßburg 1965 Basel-New York (im Druck)

20 BALDINI M. N COSTEA a.W DAMASHEK
The viability of stored human platelets.
Blood 16 1669 (1960)

21 BARKHAN P
Platelet survival studies in man with diisopropylphosphorofluoridate
Brit J Haemat. 12 25 (1966)

22 BEDSON S. P
An inquiry into the genesis of the mammalian blood platelet.
J Path. & Bact. 26 145 (1923)

23 BEIERWALTERS W H P C. JOHNSON a. A J SOLARI
Clinical use of radioisotopes.
Philadelphia Saunders 1957

24 BENHAMOU, E. et J. PUGLIESE
Les plaquettes sanguines en suspensions concentrées
Press med 70 531 (1945)
25 BENHAMOU E. et J PUGLIESE
Les plaquettes sanguines en suspensions concentrees
C R Soc Biol. 139 307 (1945)
26 BERNARD J
Anémies, leucopenies thrombopenies provoquees par des medicaments.
Press. med 70 531 (1962)
27 BERNARD J A LOVERDO B MAUPIN et J DUGAS
Note présentée au 3e Congrès de la Societée europeenne d Hématologie Rome 1951
28 BETTEX-GALLAND M a. E.F LÜSCHER
Extraction of an actinomyo in-like protein from human thrombocytes.
Nature 184 276 (1959)
29 BETTMANN J W
Drug hypersensitivity purpuras Report of a case and discussion of their mechanism
Arch. intern Med 112 840 (1963)
30 BIZZOZERO G
Über einen neuen Formbestandteil des Blutes und dessen Rolle bei der Thrombose und der Blutgerinnung.
Virchows Arch. 90 261 (1882)
31 BLEIFELD W
Lebensdauer und Abbauort von Thrombozyten in der Diagnostik von Thrombopenien.
Tag.Rhein.Westf Ges.Inn.Med Dusseldorf 18 11 1965
32 BLEIFELD W u. G GEHRMANN
Über die Bedeutung der radioaktiven Markierung von Thrombozyten mit ^{51}Cr in der Diagnostik von Thrombozytopenien.
Dtsch. med Wschr 91 1594 (1966)
33 BLEIFELD W u. G GEHRMANN
Lebensdauer und Abbauort menschlicher Thrombozyten.
11 Tg.Deutsch. Ges.f Hamatol. Okt 1965 Innsbruck
Blut 15 70 (1967)
34 BRAUNSTEINER, H
Electron microscopy of blood platelets. In. Blood Platelets, hrsg von
JOHNSON,S.A. MONTO R.W REBUCK J W and HORN R. C.
Little Brown, Boston 616 (1961)
35 BRECHER G a. E. P CRONKITE
The effect of platelet transfusion in dogs made pancytopenic by X irradiation.
N Y Str J Med 53 544 (1953)
36 BRECHER G and E P CRONKITE
Morphology and enumeration of human blood platelets.
J appl. Physiol. 3 365 (1950)
37 BREIDENBACH, H. J GRIMM und M. ZERRES
Nachweis und Verhalten thrombozytarer Antikorper bei Diagnostik und Therapie der chron. idiopath. thrombozytopenischen Purpura.
Folia Haematol. 82 86 (1964)
38 BRIZEL, H. E a G RACCUGLIA
Giant hemangioma with thrombocytopenia Radioisotopic demonstration of platelet sequestration.
Blood 26 751 (1965)
39 BROWN R. C.
Platelet labelling with ^{51}Cr for lifespan determination.
Blood 18 803 (1961)
40 CASTALDI, P A. a. B G FIRKIN
Studies of the lifespan and fate of platelets.
Austr Ann. Med. 12 333 (1963)
41 CASTALDI P A. a. B G FIRKIN
Platelet changes in coagulation
Chemical studies
Medical Research 1 35 (1962)
42 COHEN, P M H. COOLEY and F H. GARDNER
Platelet preservation II Comparison of radioactivity yields of platelet concentrates derived from blood anticoagulated with EDTA and ACD
New Engl. J Med. 273 845 (1965)
43 COHEN P F H. GARDNER, and O BARNETT
Reclassification of the thrombocytopenias by the ^{51}Cr labelling method for measuring platelet lifespan.
New Engl. J Med 264 1294 (1964)
264 1350 (1964)
44 COHEN J A. a. M.G P T WARRINGA
The fate of P^{32} labelled DFP in the human body and its use as a labelling agent in the study of the turnover of blood plasma.
J Clin Invest. 33 459 (1954)
45 COHEN P a GARDNER F H.
Platelet preservation. II Preservation of canine platelet concentrates by freezing in solutions of glycerol plasma
J Clin. Invest. 41 1 (1962)
46 CONLEY C. L.
Blood platelets and platelet transfusions.
Arch. Intern. Med. 107 635 (1961)
47 CRADDOCK C.G W S ADAMS S. PERRY a. J S. LAWRENCE
The dynamics of platelet production as studies by a depletion technique in normal and irradiated dogs.
J Lab & Clin. Med. 45 906 (1955)
48 CRONKITE, E. P
Blood platelets.
In Blood platelets hrsg. von JOHNSON S A MONTO R.W REBUCK J W and HORN R.C. Little Brown, Boston 639 (1961)
49 CRONKITE E.P V P BOND T M. FLIEDNER D A.PAGLIA and E. R. ADAMIK
Studies on the origin, production and destruction of platelets.
In Blood platelets hrsg. von JOHNSON S A.

MONTO R.W REBUCK J W and HORN R. C. Little Brown Boston, 595 (1961)
50 CRONKITE E P V P BOND J S. ROBERTSON a. D E PAGLIA
The survival, distribution and apparent interaction with capillary endothelium of transfused radiosulfate labelled platelets in rats.
J Clin. Invest 36 888 (1957)
51 CROSBY W H.
Is hypersplenism a dead issue?
Blood 20 94 (1962)
52 DAMASHEK W a. S ESTREN
The spleen and hypersplenism.
New York Grune & Stratton (1947)
53 DAMASHEK, W a. E. B MILLER
The megacariocytes in idiopathic thrombocytopenic purpura a form of hypersplenism.
Blood 1 27 (1946)
54 DAUSSET J J COLOMBANI and M. COLOMBANI
Study of leucopenias and thrombocytopenias by the direct antiglobulin-consumptionstest on leucocytes and/or platelets.
Blood 18 672 (1961)
55 DAVEY M. G
Survival and destruction of human platelets.
S. Karger Basel, New York Bibl. Haematol. fasc. 22 (1966)
56 DAVEY M. G a. H. LANDER
The behaviour of blood platelets in the first 24 hours.
Brit. J Hematol. 94 10 (1964)
57 DAVEY M. G a. H. LANDER
The labelling of human platelets with radiochromate
Austr J exp Biol. Med Sci 41 581 (1963)
58 DESAI R.G W SMALL a. L MEDNICOFF
Studies on the survival and metabolic activity of platelets in humans, utilizing radioactive phosphorus.
J Clin. Invest. 34 930 (1955)
59 DIGGS L. W a. J S. HEWLETT
Study of bone marrow from 36 patients with idiopathic hemmorrhagic (thrombopenic) pur pura
Blood 3 1090 (1948)
60 DJERASSI, J S. FARBER a. A.E.EVANS
Transfusions of fresh platelet concentrates to patients with secondary thrombocytopenia.
New Engl. J Med. 268 221 (1963)
61 DOAN C. A.
Hypersplenism.
Bull. N Y Acad. Med. 25 625 (1949)
62 DONNI A
De L origine des globules du sang, de leur mode de formation et de leur fin.
C. R. Acad. Sci. 14 366 (1842)
63 DUKE, W W
The rate of regeneration of blood platelets.
J Exper. Med. 14 265 (1911)
64 DUKE, W W
The relation of blood platelets to hemorrhagic disease Description of a method for determining the bleeding time and the coagulation time and report of 3 cases of hemorrhagic disease relieved by transfusion.
J A M. A. 55 1185 (1910)
65 EBAUGH, F G Jr, C. P EMERSON and J F ROSS
The use of radioactive chromium 51 as an erythrocyte tagging agent for the determination of red cells survival in vivo
J Clin. Invest. 32 1260 (1953)
66 EBBE S. M.BALDINI a. J DONOVAN
Comparative studies of platelet survival by different methods in the rabbit
Blood 25 548 (1965)
67 FINCH C. A.
Thrombokinetics. Diskussionsbemerkung in Blood platelets hrsg von JOHNSON S A. MONTO R.W REBUCK J W and HORN R. C. Little Brown, Boston 639 (1961)
68 FIRKIN B G
The platelet.
Austr Ann. Med. 12 261 (1963)
69 FIRKIN G B a. W J WILLIAMS
The incorporation of radioactive phosphorus into the phospholipids of human leucocytes and platelets.
J Clin. Invest 40 423 (1961)
70 FLÜCKIGER P A HÄSSIG und F KOLLER
Technique of platelet-Coombs-Test.
Acta haemat. 12, 339 (1954)
71 FLURY R. und F REUTTER
Letaler anaphylaktischer Schock während Transfusion eines Thrombozytenkonzentrates.
Schweiz. med. Wschr 96 918 (1966)
72 FONIO A.
Über fraktionierte Bluttransfusion.
Schweiz. med. Wschr 14 337 (1936)
73 FRANK E.
Die essentielle Thrombopenie.
Berl. klin. Wschr 52 454 (1915)
74 FRANK H. a. S. J GRAY
The determination of plasma volume in man with radioactive chromic-chloride
J Clin. Invest. 32 991 (1953)
75 FREIREICH, E. J., A. KLIMAN L. A. GAYDOS N. MANTEL a. E. FREI.
Response to repeated platelet transfusion from the same donor
Ann. of Int. Med. 59 227 (1963)
76 GEHRMANN G
Klinische und immunologische Aspekte des Morbus Werlhof
Dtsch. med. Wschr 91 1069 (1966)
77 GEHRMANN G
Lebensdauer und Abbauort der Erythrozyten bei hamolytischen Anämien.
Dr Alfred Huthig Verlag, Heidelberg (1964)
78 GEHRMANN G. und W BLEIFELD
Lebensdauer und Abbauort menschlicher Thrombozyten bei unterschiedlichen Thrombopenieformen.

Blut 17 266 (1968)

79 GEHRMANN G, W BLEIFELD und D KAULEN
Herzklappenfehler und Haemolyse
Kli. Wschr 44 1229 (1966)

80 GIBLET E R A G MOTULSKY F CASSERD B HOUGHTON a.C A FINCH
Studies on the pathogenesis of splenic anemia
Blood 11 1118 (1956)

81 GRAY S J a K STERLING
The tagging of red cells and plasma protein with radioactive chromium
J Clin Invest 29 1604 (1950)

82 GREGERSEN M J a R A RAWSON
Blood volume
Physiol. Rev 39 307 (1959)

83 GROSS R
Thrombozytär bedingte Blutungsübel.
Wien. klin. Wschr 78 429 (1966)

84 GROSSMAN C M., R. KOHN a. R. KOCH
Possible errors in the use of P32 orthophosphate for the estimation of platelet lifespan
Blood 22 9 (1963)

85 HARRINGTON W J V MINNICH, J W HOLLINGSWORTH a. C V MOORE
Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura
J Lab & Clin Med 38 1 (1951)

86 HARRINGTON W.J. C C SPRAGUE V MINNICH C.V MOORE R C AULVIN and R. DUBACH
Immunologic mechanisms in idiopathic and neonatal thrombocytopenic purpura.
Ann inter Med 38 433 (1953)

86a HEILMEYER L.
Blut und Blutkrankheiten
Hdbch. Inn Med 2. Bd (1951) S 894

86b HEIMPEL, H. u. W ADAN
Untersuchungen über die Lebenszeit menschlicher Blutplättchen mit radioaktivem Diisopropylfluorophosphat (DFP32)
Klin. Wschr 44 1153 (1966)

87 HEMPELMANN L. H. H LISCO and J G HOFFMANN
The acute radiation syndrome A study of nine cases and a review of the problem.
Ann. Intern. Med. 36 279 (1952)

88 HEYSSEL, R. M.
Determination of human platelet survival utilizing C14 labelled serotonin
J Clin. Invest 40 2134 (1961)

89 HEYSSEL, R. M, A. B BRILL and H. MOORE
Further experience with C14 labelled serotonin.
J Clin. Invest. 40 763 (1962)

90 HIRSCH, E. O J FAVRE-GILLY and W DAMASHEK
Thrombopathic thrombocytopenia Successful transfusion of blood platelets.
Blood 5 568 (1950)

91 HIRSCH E O a. F H. GARDNER
The transfusion of human blood platelets.
J Lab & Clin. Med 39 556 (1952)

92 HIRSCH, E. O a. F H. GARDNER
The lifespan of transfused human platelets.
J Clin. Invest. 30 649 (1951)

93 HJORT, P F a. H. PAPUTCHIS
Platelet lifespan in normal splenectomized and hypersplenic rats.
Blood 15 45 (1960)

94 HJORT P F H PAPUTCHIS a B CHENEY
Labelling of red blood cells with radioactive diisopropylfluorophosphate (DFP32) evidence for an initial release of label.
J Lab & Clin. Med 55 416 (1960)

95 HUGHES JONES N C a. L. SZUR
Determination of red cell destruction using 51Cr labelled cells.
Brit J Haemat. 3 320 (1957)

96 HUMBLE, J G
The hematological aspects of regional and total body thermotherapy
Proc roy Soc. Med 55 649 (1962)

97 JACKSON D P a. J R KREVANS
Survival of platelets transfused into thrombocytopenic recipients.
Bull. John Hopkins Hosp 107 349 (1960)

98 JANDL J H.
Sequestration by the spleen of red cells sensitized with incomplete antibody and with metallo-protein complexes.
J Clin. Invest 34 912 (1955)

99 JANDL, J H. M. S. GREENBERG R. H. YONEMOTO a. W B CASTLE
Clinical determination of the sites of red cell sequestration in hemolytic anemias.
J Clin. Invest. 35, 842 (1956)

100 JANDL, J H. J A. RICHARDSON and W B CASTLE
Destruction of red cells by antibodies in man.
I. Observation on sequestration and lysis of red cells altered by immune mechanisms.
J Clin. Invest 36 1428 (1957)

101 JULLIARD J B MAUPIN R CHARY R. THEILLEUX, P NAU et A. LOVERDO
Transfusion au lapin de leucocytes et de plaquettes du sang humain après marquage par le radiophosphore
Comp rend Soc. Biol. 146 211 (1952)

102 JULLIARD J B MAUPIN A LOVERDO J BERNARD P COLVEZ et M LECOMTE
Premiers essais de transfusion à l'homme de leucocytes et de plaquettes marquées au radiophosphore.
Press. med 60 518 (1952)

103 KAZNELSON P
Verschwinden der hämorrhagischen Diathese bei einem Fall von essentieller Thrombopenie (Frank) nach Milzexstirpation Splenogene thrombolytische Purpura.
Wien. Klin. Wschr 29 1451 (1916)

104 KISSMEYER NIELSEN F
Platelet in blood stored in untreated and

siliconed glass bottles and plastic bags. I. studies in vitro
J Clin. Path. 14 626 (1961)
105 KISSMEYER NIELSEN F
a. C B MADSEN
Platelets in blood stored in untreated and siliconed glass bottles and plastic bags. II Survival studies.
J Clin. Path. 14 630 (1961)
106 KRASSO H.
Über den Wert und die Wirkungsweise der Bluttransfusion bei der thrombozytopenischen Purpura.
Wien. Arch. Inn. Med. 14 377 (1927)
107 KREVANS, J R.
Survival of platelets transfused into thrombocytopenic recipients.
In Blood Platelets hrsg. von JOHNSON S A MONTO R.W REBUCK J W and HORN R. C. Little Brown, Boston 651 (1961)
108 KUMMER, H.
Thrombozytensuspensionen und -konzentrate
in Blutstillung, Schattauer Verlag Stuttgart 1966
109 LAMERTON L F A H PONTIFEX N M BLACKETT a K ADAMS
Effects of protracted irradiation on blood forming organs of the rat I continuous exposure
Brit J Radiol. 33 287 (1960)
110 LANDER H a. M. G DAVEY
The behaviour and survival of platelet in polycythaemia and thrombocythaemia.
Austr Ann. Med 13 207 (1964)
111 LANDER H R. L KINELOUGH a. H N ROBSON
Reduced platelet survival in patients with Starr Edwards prostheses.
Brit Med. J 5436 688 (1965)
112 LARRIEU M. J G MESHAKA J CAEN et J BERNARD
Traitement du purpura thrombopenique idiopathique
Sem Hop Paris 40 403 (1964)
113 LAWRENCE,J S a W N VALENTINE
The blood platelets The rate of their utilization in the cat
Blood 2 40 (1947)
114 LEEKSMA C H W a J A COHEN
Determination of the lifespan of human blood platelets using labelled diisopropylfluorophosphonate
J Clin. Invest 35 964 (1956)
115 LEEKSMA C H. W a. J A COHEN
Determination of the life of human blood platelets using labelled diisopropylfluorophosphonate
Nature 175 552 (1955)
116 *LE ROY G V*
Hematology of the atomic bombs casualties
Arch. Inter Med 86 691 (1950)
117 Van LOGHEM J J H. DORFMEIER a. M van der HART
Serological and genetical studies on platelet antigen (Zw)
Vox Sang 4 161 (1959)
118 LYONS W S
Desintegration of ^{51}Cr
Physical review 87 1126 (1952)
119 MARCUS A. J a. M. B ZUCKER
The physiology of blood platelets.
Grune and Stratton New York and London (1965)
120 MATHE, G H. JAMMET B PENDIC, L. SCHWARZENBERG J F DUPLAN B MAUPIN R. LATARJET M. J LARRIEU D KALIC et Z DJUKIC
Transfusions et greffes de moelle osseuse homologue chez humains irradiés a haute dose accidentellement.
Rev fr Rt Clin & Biol. 4 226 (1959)
121 MATTER M. J R HARTMANN J KANTZ A. De MARSH a C. A. FINCH
A study of thrombopoesis in induced acute thrombocytopenia.
Blood 15 174 (1960)
122 MAUPIN B
Durée de vie des plaquettes.
Rev Franc Clin. et Biol. 2 72 (1957)
123 MAUPIN B et R CHARY
Marquage par le radiophosphore des plaquettes sanguines humaines Transfusion à l homme et a l animal
Soc franc. Hematologie (1951)
124 MAUPIN B et A LOVERDO
Marquage in vitro par l or colloidal radioactiv des leucocytes et des plaquettes du sang humain
C. R. Soc. Biol. 152 277 (1958)
125 MAUPIN B A LOVERDO R THEILLEUX, V BAUDOT a. E. LEMOINE
Studies on human platelets labelled in vitro by radiochromate
Proc VI[th] Congress of the Intern. Soc of Blood transfusion (1958)
126 Mc FARLAND W N B GRANVILLE a. W DAMASHEK
Autologous bone marrow infusion as an adjunct in therapy of malignant disease
Blood 14 503 (1959)
127 Mc ILVANIE S
Glass vs. plastic containers for platelet transfusion
Proc. Congr Int Soc. Blood Transf Boston (1956) Karger Basel and New York (1958)
128 MEIERS H. G GEHRMANN und W GAHLEN
Antikorper bedingte thrombozytopenische Purpura nach Phenylbutazon.
Dtsch. med Wschr 88 850 (1963)
129 MINOT G R. and R J LEE
The blood platelets in hemophilia
Arch. Intern. Med 18 474 (1936)
130 MOLLISON P L. and N VEALL
The use of the isotope ^{51}Cr as a label for

red cells.
Brit J Haematol. 1 62 (1955)
131 MORGAN M. C. R. P KEATING and E. H. REISNER
Survival of radiochromate-labelled platelets in rabbits.
J Lab Clin. Med 46 521 (1955)
132 *MORGAN M. C. R. P KEATING and* E. H. REISNER
Labelling of rabbit platelets with jodine 131
Proc. Soc.Exper Biol. & Med 85 420 (1954)
133 *MUELLER, J F*
Pathologic physiology of mammalian blood platelets utilizing P^{32} tagged rabbit platelets.
Proc Soc.Exp Biol. (N Y) 83 557 (1953)
134 MURPHY E A. a. J F MUSTARD
Platelet economy during moderate and intensive dicoumarol therapy
Lancet 2 960 (1961)
135 MURPHY E A a J F MUSTARD
Dicoumarol therapy and platelet turnover
Circulation Res. 9 402 (1961)
136 MURPHY E A. J F MUSTARD and G ROBINSON
The pattern of platelet destruction
Blood 20 765 (1962)
137 MUSTARD J F
Platelet economy (platelet survival and turn over)
Brit J of Haematol. 12 1 (1960)
138 MUSTARD J F H D GREISSINGER G.A ROBINSON H.C.ROWSWELL E. A. MURPHY M. Mc. CARTER and P RIDELL
Factors influencing the fate of the platelets.
Blood 20 765 (1962)
139 MUSTARD J F a. E. A. MURPHY
Blood platelet economy during moderate and intensive heparin therapy
Blood 22 1 (1963)
140 MUSTARD J F a. C B V WALKER
The influence of blood collecting on platelet numbers during blood storage
Brit J Haematol. 3 50 (1957)
141 NAJEAN Y a. N ARDAILLOU
The physiopathological study of platelets by means of radioisotopes.
Hrsg. von E. SZIRMAI in Nuclear Hematology Academic Press New York and London 41 (1965)
142 NAJEAN Y et N ARDAILLOU
Etude de la duree de vie des plaquettes marquees au radiochrome dans le purpura thrombopenique
Semain Hop Paris 40 395 (1964)
143 NAJEAN Y N ARDAILLOU L CAEN M. J LARRIEU a. J BERNARD
Survival of radiochromium labelled platelets in thrombocytopenias.
Blood 22 718 (1963)
144 NAJEAN Y M. J LARRIEU et J BERNARD
Survie des plaquettes marquees au radiochrome
Nouv Rev franc. Hemat 1 36 (1961)
145 NAJEAN Y MJ LARRIEU et J BERNARD
Technique d etude de la survie des plaquettes par la méthode de marquage au radiochrome
Rev franc Etud. clin. biol. 4 1071 (1959)
146 *ODELL, T T*
Platelet labelling with radioisotopes and in vivo platelet survival.
In.Blood platelets hrsg. von JOHNSON S A. *MONTO R W REBUCK J W and HORN* R. C Little Brown Boston 643 (1961)
147 ODELL, T T
Production lifespan and fate of blood platelets. Studies with radioisotope labelling techniques.
Proc. 6th Congr Int. Soc. Haemat Boston, 294 (1956)
148 ODELL T T a. B ANDERSON
Production and lifespan of platelets.
Hrsg. F STOHLMANN Jr New York Grune & Stratton in The Kinetics of Cellular Proliferation 278
149 ODELL T T F N GAMBLE a J FURTH
Lifespan of naturally labelled platelets of rats
Fed Proc 12 398 (1953)
150 ODELL T T a R M KNISELEY
The origin lifespan regulation and fate of blood platelets.
Progress in Hematology Vol. 3 203 New York and London Grune & Stratton (1962)
151 ODELL T T F G TAUSCHE a J FURTH
Platelet lifespan as measured by transfusion of isotopically labelled platelets into rats.
Acta heamat 13 45 (1955)
152 ODELL T T F G TAUSCHE a. J FURTH
Lifespan of in vivo labelled transfused platelets
Fed Proc 13 440 (1954)
153 ODELL T T F G TAUSCHE a W D GUDE
Uptake of radioactive sulfate by elements of the blood and the bone marrow of rats
Amer J Physiol. 180 191 (1955)
154 O NEILL B a. B FIRKIN
Platelet survival studies in coagulation disorders thrombocythaemia and condition associated with atherosclerosis.
J Lab Clin Med. 64 188 (1964)
155 OWEN C. A. Jr J L. BOLLMANN a. J H GRINDLAY
Radiochromium-labelled erythrocytes for the detection of gastro intestinal hemorrhage
J Lab Clin. Med 44 238 (1954)
156 OZGE A. M. BALDINI a. R GOLDSTEIN
Effect of plastic and glass surfaces on clot retraction and serotonin uptake of plate-

letrich plasma stored at 4° C.
J Lab Clin Med 63 378 (1964)
157 PARKER WILLIAMS E J P COHEN P WATROUSE a F H CARDNER
Platelet preservation studies using platelets labelled with C^{14} 5 hydrooxytryptamine
J Clin Invest 42 963 (1963)
158 PERMAN V E P CRONKITE V P BOND a D K SORENSEN
The regenerative ability of haemopoietic tissue following lethal x irradiation in dogs.
Blood 19 724 (1962)
159 PFEIFFER E F SPIELMANN u. H DITSCHUNEIT
Die klinische Bedeutung des Nachweises von Antikorpern gegen Thrombozyten bei thrombozytopenischer Purpura
Dtsch. med Wschr 82 735 (1956)
160 PFISTERER H. S. THIERFELDER C. THIEDE u. W STICH
Vergleichende Untersuchungen über erythrocytar (ABO) kompatible und inkompatible Thrombozytentransfusionen beim Menschen.
Klin. Wschr 44 613 (1966)
161 PISCIOTTA A. V M. STEFANINI a W DAMASHEK
Studies on platelets. X. Morphologic characteristics of megacaryocytes by phase contrast microscopy in normals and in patients with idiopathic thrombocytopenic purpura.
Blood 8 703 (1953)
162 POLLYCOVE M. G DALSONTO a. J H. LAWRENCE
Simultaneous measurement of erythrocyte leucocyte and platelet survival with diisopropylfluorophosphate 32 (DFP32)
Clin Res. Proc. 4 80 (1960)
163 PONTIFEX, A. H a L.F LAMERTON
Effects of protracted irradiation on the blood forming organs of the rat. Part II. Divided doses.
Brit. J Radiol. 33 736 (1960)
164 RACCUGLIA G a. C.J D ZARAFONETIS
Platelets and pharmacologic compounds in radiation-induced haemorrhagic diathesis.
Amer J med Sci. 244 152 (1962)
165 RAJEWSKI B
Strahlendosis und Strahlenwirkung
Thieme-Verlag Stuttgart (1954)
166 RAPOPORT S
Dimensional, osmotic and chemical changes of erythrocytes in stored blood. I. Blood preserved in sodium citrate neutral and acid citrate-glucose (ACD) mixtures.
J Clin. Invest 26, 591 (1947)
167 READ R. C.
Studies on red cell volume and turnover using radiochromium. Description of a new "closed method of red cell volume measurement.
New Engl. J Med. 250 1021 (1954)
168 REISNER, E. H. R. P KEATING C. FRIESEN a. E. LOEFFLER.
Survival of sodium chromate 51 labelled platelets in animals and man.
Proc. VI[th] Intern. Congr Soc. Haemat. Boston 1956, New York and London, Grune and Stratton 292 (1953)
169 ROBERTSON J S W L MILNE a. S H COHN
Labelling and tracing rat blood platelets with chromium 51
Radiois. Conf Proc. of the 2[nd] Conf., Oxford New York Academic Press, Inc. 205 (1954)
170 ROBINSON G A. P RIDELL A. Mc CARTER H. C. ROWSWELL a. J F MUSTARD
Thrombin and intravascular platelet thrombi.
Blood 20 789 (1962)
171 Van ROOD J J J G EERMISSE a. A Van LEEUWEN
Some studies on the effect of circulating thrombocyte-antibodies on the lifespan of thrombocytes.
Proc. VI[th] Congr Europ Soc. Haematol. Copenhagen (1957)
Basel, New York Karger 821 (1953)
172 ROY A. Y J DJERASSI H.HEITLICH a. S FARBERS
Effects of escherichia coli endotoxin on rat platelets.
Amer J Physiol. 203 296 (1962)
173 SEIDL, S
Die Thrombozytentransfusion.
Dtsch. med. Wschr 92 79 (1967)
174 SHULMAN N R
Immunoreactions involving platelets I Asteric and kinetic model for formation of a complex from a human antibody quinine as haptene and platelets and for fixation of complement by the complex.
J Exper Med. 107 665 (1958)
175 SHULMAN N R.
Immunoreactions involving platelets:
III. Quantitative aspects of platelet agglutination, inhibition of clot retraction and other reactions caused by the antibody of quinine purpura.
J Exp Med. 107 197 (1958)
176 SHULMAN N R.
Immunoreactions involving platelets
IV Studies on the pathogenesis of thrombocytopenia in drug purpura using test doses of quinidine in sensitized individuals their implications in idiopathic thrombocytopenia.
J Exp Med. 107 711 (1958)
177 SHULMAN N R., R. H LEITNER a. M C. HILLER
Immunoreactions involving platelets: V Post transfusion purpura due to a complement fixing antibody against a genetically controlled platelet antigen. A proposed mechanism for thrombocytopenia and its relevance in

autoimmunity
J Clin. Invest 40 1957 (1961)
178 SHULMAN N R R H ASTER
H A PEARSON a M C. HILLER
Immunreactions involving platelets VI Reactions of maternal isoantibodies reponsible for neonatal purpura Differentiation of a second platelet antigen system.
J Clin. Invest 41 1059 (1962)
179 SHULMAN N R. V MARDER
a. R. S WEINRACH
Similarities between known antiplatelet antibodies and the factor reponsible for thrombocytopenia in idiopathic purpura.
Ann. N Y Acad. Sc. 124 499 (1965)
180 SHULMAN N R, R. S WEINRACH,
E P LIBRE a. H. L. ANDREWS
Role of reticuloendothelial system in pathogenesis of idiopathic and thrombocytopenic purpura.
Trans. Assa. Am. Physic. 78 274 (1965)
181 SHOUSE S S S L WARREN
a. G H WHIPPLE
Aplasia of marrow and fatal intoxication in dogs produced by roentgen irradiation of all bones.
J Exp Med. 53 421 (1931)
182 SMALL W M. BALDINI N COSTEA
a. W DAMASHEK
The in vitro uptake of ^{51}Cr by human platelets.
Clin. Res. 7 210 (1959)
183 STEFANINI, M. a. J B CHATTERJEA
Rate of platelet survival in thrombocytopenia.
J Clin. Invest. 30 676 (1961)
184 STEFANINI M. J B CHATTERJEA
W DAMASHEK C S WELCH
a. O SWENSON
Studies on platelets III The absence of selective sequestration" and destruction of platelets by the spleen in idiopathic thrombocytopenic purpura.
Blood 7 289 (1952)
185 STEFANINI, M. J B CHATTERJEA
W DAMASHEK, L. ZANNOS
a. *E P SANTIAGO*
Studies on platelets. II. Effect of transfusion of plateletrich polycythemic blood on platelets and hemostatic function in "idiopathic and "secondary" thrombocytopenic purpura.
Blood 7 53 (1952)
185a STEFANINI, M. a. W DAMASHEK
Collection preservation and transfusion of platelets with special reference to factors affecting the survival rate and the clinical effectiveness of transfused platelets.
New Eng. J Med. 248 797 (1953)
186 STEFANINI M. W DAMASHEK
a. E ADELSON
Platelets. VII Shortened platelet survival time and development of platelet agglutinins following multiple platelet transfusions.
Proc. Soc. exp Biol. 80 230 (1952)
187 STEFFEN C.
Untersuchungen über die Verwendung der Antihumanglobulin - Ablenkungsmethode zum Nachweis von Thrombozytenantikörpern.
Wien. Zschr inn. Med. 36 246 (1955)
188 STERLING K a. S J GRAY
Determination of the circulating red cell volume in man by radioactive chromium.
J Clin. Invest. 29 1614 (1950)
189 THIERY J P et M. BESSIS
La genèse des plaquettes sanguines a partir des *mégacaryocytes observee sur la cellule* vivante.
C. R Acad Sci. 242, 290 (1956)
190 TOCANTINS L. M.
Experimental thrombocytopenic purpura. Cytological and physiological changes in the blood.
Ann intern. Med 9, 838 (1936)
191 VODOPICK N A. a. R M. KNISELEY
Sulfur 35 studies in man Platelet survival and plasma and urinary radioactivity assayed by beta liquid scintillation spectometry
J Lab Clin. Med 62 109 (1963)
192 WALD, N G E THOMA a. G O BROWN
Hematologic manifestations of radiation exposure in man
Progr haemat. Vol. 3 1 Grune and Stratton New York
193 WALDMANN T A.
Gastrointestinal protein loss demonstrated *by ^{51}Cr labelled albumin.*
Lancet II. 121 (1961)
194 WAGNER H. N Jr., J G Mc AFEE
a. J W WINKELMANN
Splenic disease diagnosis by radioactive scanning.
Arch. Int Med. 109, 77 (1962)
195 WEBER, A J
Two populations of platelets.
Nature 205 1332 (1965)
196 WEISSBACH, H. a. R. G REDFIELD
Studies on the uptake of serotonin by *platelets.*
In. Blood Platelets hrsg. von JOHNSON S A. MONTO R.W REBUCK J W a.HORN R.C. Little Brown, Boston 393 (1961)
197 WERLHOF
Disquisito medica et physiologica de variolis et anthracibus.
Hannover 1735
198 *WOODS, M.C. F N GAMBLE J FURTH*
a. R. R. BIGELOW
Control of the postirradiation hemorrhagic state by platelet transfusions.
Blood 8 545 (1953)
199 ZUCKER, M.B L. HELLMANN
a. B ZUMOFF
Rapid disappearance of C^{14} labelled serotonin from platelets in patients with car

cinoid syndrome
J Lab Clin. Med 63 137 (1964)
200 ZUCKER M B A B LEY
a. K MAYER
Studies on platelets lifespan and platelets
depots by use of DFP^{32}
J Lab Clin. Med 58 405 (1961)
201 ZUCKER M. B B ZUMOFF
a. L. HELLMANN
Rapid exchange of platelet ^{14}C labelled
serotonin in patients with functioning car
cinoid tumors.
Blood 20 764 (1962)

Acta Medica Scandinavica

Supplementum 499

Studies on the pathogenesis of post-traumatic fat embolism

CONTENTS

ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 499

Studies on the pathogenesis of post-traumatic fat embolism

CONTENTS

FROM THE DEPARTMENT OF GERIATRICS II, VASA SJUKHUS, THE DEPARTMENT OF SURGERY I, THE DEPARTMENT OF MEDICINE II AND THE CENTRAL LABORATORY, SAHLGRENSKA SJUKHUSET, UNIVERSITY OF GOTEBORG, GOTEBORG, SWEDEN

I. THE CHEMICAL COMPOSITION OF THE FAT EMBOLI IN THE POST ABSORPTIVE DOG

BY

J. KERSTELL, B. HALLGREN, C. M. RUDENSTAM AND A. SVANBORG

Abstract. Pulmonary fat emboli were isolated in 7 post absorptive dogs after bone fracture. The emboli consisted mainly of triglycerides. The fatty acid composition of these triglycerides was compared with that of plasma and bone marrow triglycerides. The triglycerides in the emboli were similar to those from bone marrow but differed from those in plasma.

These observations indicated that the bone marrow fat is the source of the embolised fat in the lungs in the case of trauma.

It was also concluded from the literature that fat emboli occur in other non traumatic conditions and that there must exist other possibilities for the formation of fat emboli.

Fat embolism is used both to denote a patho anatomical condition and to describe a clinical syndrome. Patho anatomically, fat droplets appear in the small vessels where they can be stained with fat stains or osmic acid. The clinical syndrome includes various signs attributed to disturbed blood flow in different organs. The patho anatomical changes are commonly seen in individuals subjected to skeletal trauma, but the condition is also found after soft tissue injury and in various non traumatic conditions. The emboli are usually found in the lungs but to a lesser extent also in the systemic circulation. The clinical syndrome has been observed mostly after fractures.

The first case of fat embolism was reported by Zenker 1862 (60). Today, one century later, the main problems concerning fat embolism are still essentially unsolved, namely the genesis of the emboli, how the appearance of fat globules in the vessels is connected with the clinical syndrome and how to diagnose and treat the condition.

For the genesis of the emboli two main theories have been discussed: the emboli originate from the traumatized adipose tissue, especially the bone marrow, or they are formed by alterations in the physical state of the blood lipids. In addition, other theories which can be considered as modifications of these two main theories have also been presented. Many investigations have been carried out in order to elucidate the genesis of the emboli (for review see Bergentz (4), Sevitt (51), de Ruiter (50) and Bergentz (5)). In most previous investigations the results were based on histological quantitation of pulmonary fat embolism seen in various conditions. Qualitative chemical analyses of the fat emboli have not previously been performed.

The fatty acid composition of the triglycerides in the adipose tissue seems to differ somewhat in different regions, presumably due to an adaptation to differences in temperature in various parts of the body (31). Comparisons between the triglycerides in subcutaneous fat and in plasma have shown obvious differences (14, 30). An attempt to elucidate the pathogenesis of post traumatic fat embolism on the basis of this finding was made in our laboratory (24). This study showed differences in the fatty acid composition in plasma and bone marrow triglycerides in dogs. The fatty acid composition of the triglycerides from lung, plasma and bone marrow was compared in dogs with and without

fractures The contribution of lung tissue triglycerides however, made the results inconclusive A method for the isolation of the fat emboli by retrograde perfusion of the pulmonary circulation was therefore developed (28) The preliminary results of experiments using this method showed a striking similarity between bone marrow and emboli triglyceride fatty acid composition

The present report includes further qualitative and quantitative analyses of pulmonary fat emboli after fractures in fasting dogs The results obtained in the analyses of the emboli were compared with those from plasma and bone marrow

MATERIAL AND METHODS

Experiments were carried out on 15 dogs weighing 12–30 kg Five of the experiments performed with the present technique have been in a preliminary report (28) After an overnight fast the dogs were anesthetized intravenously with pentobarbital sodium (15 mg/kg body weight)

In dogs A1–A7 bilateral fractures of the femoral diaphyses were produced using a pair of pipe tongs Dogs A C1–A C5 were used as non traumatized controls During the period after the trauma the dogs layed on a warm table and the body temperature was kept at or just above the normal The fat emboli were isolated 3–4 hours after the fracture or at corresponding times

The influence of trauma on the composition of the bone marrow fat triglycerides was studied in dogs Z1–Z3 Bone marrow biopsies were taken from both femoral diaphyses immediately after the anesthesia thereafter fractures were produced After 3 hours new biopsies were taken from the femoral diaphyses No perfusion of the pulmonary vessels was made in these dogs

In two further dogs not included in the present investigation control of the analytical methods was performed (Table I and II)

The pulmonary fat emboli were washed out by retrograde perfusion of the pulmonary vessels Thereafter the emboli were isolated from the effluent perfusate by a filtration and centrifugation technique The chemical composition of the emboli was determined as was the composition of plasma and bone marrow lipids

The perfusion of the pulmonary vessels

The method for the retrograde perfusion of the pulmonary vessels was essentially the same as that described previously (24) but has been somewhat improved in several steps For this reason a detailed description is given in the present paper

The dogs were given suxamethon (Celocurin® Vitrum Sweden) 0 5 mg/kg bodyweight intravenously and thereafter intubated and ventilated with O_2+N_2O in an Aga spiro pulsator A transverse thoracotomy was performed Two ribs and the sternum were divided The right middle lobe of the lung was removed and used as a control lobe for the histological examinations The perfusion of the pulmonary vessels was carried out in two steps At first, the right lower lobe was perfused selectively and then the remaining three main lobes were perfused simultaneously (total lung perfusion)

The selective perfusion of one lung lobe

Polyethylene catheters were introduced into the main pulmonary artery branch and into a vein branch of the lower right lobe of the lung The lobe was perfused in the retrograde direction with 150 ml of isotonic sodium citrate solution pH 7 5 37 °C The perfusion pressure was kept at 50–60 cm of water The perfusate from the pulmonary artery branch was passed through a glass tube with the tip 40 cm below the heart level The lobe was extirpated after the perfusion

The perfusion of the remaining lobes

Catheters were introduced into the main pulmonary artery and into the left atrium Five ml of the sodium citrate solution was then injected into the pulmonary artery which was thereafter ligated proximal to the catheter

The heart was clamped at the level of the atrioventricular groove so that the pulmonary circulation was isolated The aorta was clamped just above the diaphragm and at the arch The remaining lung tissue was then perfused in the retrograde direction with 500 ml of the citrate solution After the perfusion the lung lobes were removed

Remarks on the perfusion step

The time from the start of the thoracotomy until the perfusions were finished was about 20 min The selective perfusion of the right middle lobe was carried out if something should fail during the technically more complicated total lung perfusion The citrate solution was injected into the pulmonary artery to avoid intravascular coagulation The aorta was clamped in order to diminish the leak through the bronchial arteries This leak was generally small as an average 90 per cent of the influent perfusates was recovered from the left atrium in the 34 dogs included in these investigations (35) However in a few dogs 30–40 per cent of the perfusion solution was lost as pulmonary oedema and by leak through the bronchial arteries

The isolation of the emboli from the effluent perfusates

The effluent perfusates from the catheters in the artery branch of the right middle lobe and in the pulmonary artery were passed through nylon filters with a pore size of 28μ

The filters were washed repeatedly with 4–5 ml of the citrate solution The perfusates were then centrifuged at 1400 *g* for 20 minutes at +5 C and were thereafter passed through nylon filters of the same kind The corpuscular pellets appearing at the bottom of the centrifuge tubes were discharged

Remarks on the isolation of the emboli from the effluent perfusates

Fat emboli passing the first filter were aggregated to a lipid layer on the surface during the centrifugation at low temperature This lipid layer was then isolated by a second filtration

The plasma lipoproteins which floated to the surface together with the emboli passed both the filters Proof of this was the observation that only trace amounts of protein were present in the emboli fraction (27) and that ^{14}C labelled chylomicrons were found to pass the filters (34)

Blood and bone marrow sampling

Blood sampling was performed through catheters in one brachial artery before the trauma and before the perfusion of the pulmonary circulation Thirty—40 ml of blood were taken for each sample

Bone marrow biopsies were performed from the femoral diaphyses The femoral diaphyses (controls) or the two diaphyses fragments (traumatized animals) were dissected free In the controls, the diaphyses were divided with a pair of tongs The medullar fat was scraped out of the medullar cavities from the isolated diaphysis fragments Liquid fat in the femoral fragments found in the traumatized dogs was included in the bone marrow specimens The fat rich parts of the specimens were dissected free from connective tissue and in most cases also washed with saline at room temperature in order to remove impurities such as blood cells In dogs Z1–Z3 biopsies before fracture were taken through a hole 0 5 cm in diameter in the femoral diaphyses made with an osteotome

Chemical methods

The lipids in the filter fractions, the remaining perfusate the plasma fractions and the bone marrow fraction were extracted using methanol chloroform 1/2 (v/v) The perfusate and plasma fractions were lyophilized before the lipid extractions The extracts were washed repeatedly with 1 per cent saline

The triglycerides were determined according to Carlson (11) the total phospholipids according to Svanborg & Svennerholm (53) and total

cholesterol according to Zlatkis Zak & Boyle (61) The triglycerides were separated from the other lipid components by thin layer chromatography on silicic acid Light petroleum+acetic acid+methyl ethyl ketone 45 0 5 4 (v/v/v) was used as solvent The triglyceride fatty acid composition was analysed using gas liquid chromatography, essentially according to Hallgren Stenhagen Svanborg & Svennerholm (29)

Gas liquid chromatography A Perkin Elmer gas chromatograph model 116 was used with a flame ionization detector The columns were made of aluminium tubes with an inner diameter of 4 mm and a length of 2 m Column I 5% w/w SE 30 coated on 80-100 mesh Gas Chrom Q (Applied Science Lab) operated at 215° Injection temperature 270 C Column II 15% w/w DEGS (diethylene glycol succinate) coated on 80-100 mesh Gas Chrom P operated at 190 C Injection temperature was 240 C Helium was used as the carrier gas The peaks were identified from log retention diagrams

The blood hematocrit values in all blood samples were determined and hemoglobin was measured as oxyhemoglobin or cyanmethemoglobin in blood and perfusates

Remarks on the chemical analyses

The fatty acid determination was carried out in the filter and filtrate fractions from the perfusate obtained from the right lower lobe of the lung in dog A1 and from the remaining 3 main lobes in dogs A2-A7 In dogs A2-A7 only quantitative estimations of cholesterol phospholipids and triglycerides were made in the fraction from the perfusate of the right lower lobe For technical reasons no 'total lung perfusion was performed in dog A1 For the quantitative estimations of fat emboli and perfusates, the total amount of lipids in all filter fractions and perfusates is given For the estimation of the fatty acid composition of the triglycerides the mean values for the fractions analysed are presented The present quantitation of triglycerides in the filter and filtrate fractions differ from the quantitation made earlier The figures for the filter fractions include the triglycerides extracted from the walls of the filter funnels which was not the case in the earlier preliminary report (28)

In the present investigation further attempts were made to quantitate the embolised fat The analyses showed that the small amounts of fat retained on the filter funnels had the same triglyceride composition as that on the filters (Table I) Therefore the amount of fat of the filter funnels was included in the figures for the emboli fraction Furthermore, at the calculation of the volume of the filtrate the approximate volume of solution discharged together with the corpuscular pellet was included in the present figures of filtrate volumes

In order to estimate the errors in the whole analytical procedure, duplicate samples were taken in one non traumatized dog not included in the present study The samples were taken from plasma on two occasions from bone marrow from subcutaneous inguinal fat tissue and one from a filtrate fraction The differences between the duplicates were very small (Table II)

Table I The percentages of C18 1 C18 2 C20*p* and C22*p* fatty acids in triglycerides extracted from the filter the funnel walls and from bone marrow and plasma in one dog

	Filter fraction A	Filter funnel A upper part	Filter funnel A lower part	Filter fraction B	Filter funnel B upper part	Filter funnel B lower part	Bone Marrow	Plasma mean value Plasma I and II
18 1	45 5	43 3	46 0	45 8	43 7	44 1	42 8	28 7
18 2	10 3	11 1	11 4	10 2	11 0	10 0	9 1	13 2
20p	1 1	0 9	0 9	0 7	1 1	1 5	– 4	9 6
22p	0 5	0 6	0 2	0 4	0 4	0 8	0 7	3 5

Table II The fatty acid composition of triglycerides in duplicate extracts (A and B) from plasma on two occasions from bone marrow subcutaneous inguinal fat tissue and filtrate fraction in one dog

Fatty acid	Plasma 1		Plasma 2		Bone Marrow		Subcut fat tissue		Filtrate	
	A	B	A	B	A	B	A	B	A	B
12	0.5	0.6	0.4	0.4	0.2	0.3	2.1	2.2	0.6	0.6
14	2.4	2.4	2.3	2.1	2.4	2.6	5.5	5.6	2.7	2.5
15	1.2	1.2	1.1	1.1	0.7	0.5	0.5	0.6	1.0	0.9
16:0	23.6	23.6	21.6	21.4	21.5	21.6	20.6	20.7	24.3	24.3
16:1	2.9	2.9	2.8	2.7	4.0	4.1	3.0	3.0	3.1	3.1
17	2.1	2.1	2.4	2.4	1.1	1.0	0.8	0.8	2.3	2.3
18:0	8.5	8.7	10.5	10.7	7.0	6.6	10.9	10.8	9.4	9.7
18:1	36.1	36.1	34.4	34.4	48.2	48.3	40.9	41.0	36.9	36.9
18:2	15.0	14.5	14.7	14.5	10.3	10.2	9.7	9.7	12.3	12.1
19	0.5	0.5	0.5	0.5	0.2	0.2	0.2	0.2	0.3	0.4
20:0	0.3	0.3	0.5	0.5	0.4	0.4	0.7	0.6	0.3	0.3
20:1	1.2	1.2	1.2	1.4	1.8	1.7	2.7	2.6	0.9	1.0
20p	2.7	2.6	3.5	3.4	0.4	0.5	0.1	0.1	1.9	1.9
22:0	2.0	2.4	3.1	3.5	1.2	1.5	1.3	1.2	3.2	3.2
22:1	0.1	0.1	0.3	0.3	0.4	0.4	1.0	0.9	0.4	0.4
22 p	0.8	0.8	0.7	0.7	0.2	0.2	0	0	0.4	0.4

Histological methods

All lung lobes were placed in 10 per cent formalin solution Frozen sections were cut out from the lung specimens and were prepared for microscopy and stained with osmium according to Romeis (49) The thickness of the specimens was 15–20μ From the control lobe 3 sections (about 9 cm²) and 2 sections (about 6 cm²) from the perfused lobes were analysed The area of the sections was measured with a planimeter The percentage of emboli left in the perfused lobes was calculated by comparison of the mean value for the number of emboli/cm in the perfused lobes with that in the control lobe

Remarks on the histological methods

Only intravascular droplets were considered as emboli When many droplets were found in single file close together they were counted as one embolus Minute fat droplets (<20μ) were not included in the calculations As no essential differences between the yield in perfusion existed between the lobe perfused singly and those included in the total lung perfusion, all perfused lobes were included in the calculations

Terms used

Plasma I	denotes the plasma samples before the trauma
Plasma II	denotes the plasma samples before the perfusion procedure started
Emboli	*denotes the filter fractions*
Filtrate	denotes the lipids in the perfusates passing both filters
Bone marrow	denotes the bone marrow specimen

From the hematocrit hemoglobin values as well as from the phospholipid values the theoretical amount of plasma in the perfusates was calculated The amount of plasma triglycerides in the perfusate was thereafter calculated The individual fatty acids are listed after the number of carbons The number of double bonds are given for C16 C18 C20 and C22 fatty acids by the figure after the colon

p denotes polyunsaturated fatty acids All individual fatty acid values are means of duplicates expressed in weight percentages of total fatty acid methyl esters

Statistical methods

Standard statistical methods and terms were used (52)

In the tables mean is denoted M and standard error of the mean SE

Differences in fatty acid composition were analysed by two sided analysis of variances after arc sin transformations of the percentage values

The model for a difference was

$$D_{ij} = \mu + A_i + F_j + Z_{ij}$$

where D_{ij} denotes the difference μ the mean value A_i the animal factor F_j the fatty acid factor and Z_{ij} a random factor As the percentage values are not independent variables

Table III Triglycerides (TG), cholesterol (Chol) and phospholipids (Lip) in the plasma (mg/100 ml) before the fracture (Plasma I) and before the perfusion (Plasma II). The lipid values for the emboli and filtrate are given as mg in the total fractions

Traumatized dogs	Plasma I			Plasma II			Emboli			Filtrate			Filtrate Amount plasma TG calculated from	
	TG	Chol	Lip	TG	Chol	Lip	TG	Chol	Lip	TG	Chol	Lip	Hb	Lip
A1*	29	228	394	20	221	401	14	0.4	0.8	20	49	72	4	4
A2	[illegible]	182	170	17	213	187	22	2.4	1.8	1	[illegible]	70	5	6
A3	35	192	342	32	190	308	10	0.2	2.3	2	7	33	3	3
A4	[illegible]	137	269	ta	148	280	157	1.1	0.5	40	124	212	—	—
A5	30	97	167	20	98	167	100	1.0	1.6	10	35	63	6	8
A6	52	209	368	35	228	360	15	1.6	2.7	11	84	139	14	13
A7	51	322	398	39	101	352	4	0.1	1.0	20	80	148	18	16
Mean ∓ SE	37∓5	193∓29	301∓40	30∓4	182±19	293∓31	52∓21	1.0∓0.3	1.6∓0.3	10∓5	62∓15	105∓24	8∓3	8∓2
Control dogs														
A C1	23	140	280	32	147	269	0.9	0.7	0.7	0	73	130	—	—
A C2	48	193	333	49	209	380	0.6	0.6	1.6	13	65	124	10	16
A C3	54	190	360	44	214	392	0.4	0	1.5	4	23	35	0	4
A C4	30	135	258	30	148	278	0.2	0.4	0.4	3	32	53	0	0
A C5	40	151	267	36	171	273	0.7	0.4	0.4	6	47	81	10	11
Mean∓SE	39∓6	161∓12	301∓21	38∓4	178∓14	318∓28	0.5∓0.1	0.4∓0.1	0.9∓0.3	7∓2	48∓10	84∓18	8∓2	9∓3

*The emboli and filtrate values represent 4 times the values obtained in the single perfused lung lobe

(they must add up to 100 per cent) a reduction of the degrees of freedom was also necessary

If the analysis of variances gave significant differences between fatty acid percentages Tukey s method was adapted to find out which fatty acid percentages differed significantly

When comparing the similarity of the fatty acid composition in the triglycerides between emboli vs bone marrow (relation 1) and between emboli vs plasma fraction (relation 2) the following statistical analyses were made

For both relations the following data were calculated the correlation coefficient (r) the regression equation ($y_{\bar{x}}=a+bx$) and the residual standard deviation ($s_{yx}=s_y\sqrt{1-r^2}$) which is a measure of agreement
Providing that,

$$r_1 > r$$
$$|b_1-1| < |b-1|$$
$$|a_1| < |a|$$
$$s_{(yx)1} < s_{(yx)2}$$

the two residual variances were compared by Snedecors F test and also here a reduction of the degrees of freedom was necessary

$$F=\frac{s^2_{(yx)2}}{s^2_{(yx)1}}$$

$$df_1=df=n-3$$

When the residual variances differed significantly ($p<0\ 01$) relation 1 was considered closer than relation 2

RESULTS

All dogs tolerated the trauma well At necropsy liquid fat was found within or in association to the femoral fragments in most traumatized dogs No such liquid fat was found in the non traumatized animals

The histological examinations showed 23 fat emboli per cm of *lung tissue in the control* lobe in dog A1 1–3 emboli in dogs A2–A7 and about one embolus in dogs Z1–Z3 The perfused lobes showed at an average 60 per cent of the number of emboli in the non perfused control lobes In dogs A2, A5 and A6 no emboli were left in one to three lobes after the perfusion

In the controls only a few emboli were observed in the control lobes, and in the perfused lobes generally, no emboli were observed

In the traumatized dogs a layer of fat droplets was seen on the filters No such layer was observed in the non traumatized controls Table III shows the amount of triglycerides cholesterol and phospholipids in the different fractions in all dogs The amount of triglycerides was considerably higher in the emboli fractions in all the traumatized dogs than in the controls More than 50 per cent of the emboli fat was caught on the second filter In all animals the amount of triglycerides found in the *filtrates* was in the magnitude of that calculated to be derived from the plasma in the perfusates or higher (traumatized dogs) A moderate decrease of the plasma triglycerides was found in the *traumatized dogs* The amount of *emboli trigly*cerides was not correlated to the drop in the plasma triglycerides

The fatty acid composition differed in plasma and bone marrow fat both in traumatized and non traumatized animals (*Table IV and V*) The analyses of variances were carried out in the control animals and showed that the difference between plasma and bone marrow triglycerides in the fatty acid composition was statistically significant ($p<0\ 001$) The trauma caused no significant alterations in the fatty acid composition of the bone marrow triglycerides in dogs Z1–Z3 (Table VI)

In Table IV and fig 1 the fatty acid composition of the triglycerides in the different fractions in the traumatized dogs is given The analyses of variances showed a significant difference between the fatty acid composition in emboli and plasma triglycerides but no difference between emboli and bone marrow triglycerides (Table VII) There were significantly lower percentages of C18 1 and significantly higher percentages of C18 2 C20*p* and C22*p* fatty acids in plasma than in emboli triglycerides A striking similarity between the *composition of emboli and bone marrow*

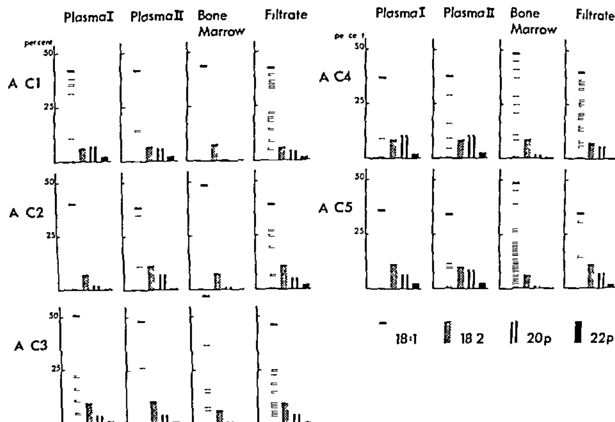


Fig 2 The percentages of 18 1 18 2 20*p* and 22*p* fatty acids in triglycerides in plasma at corresponding time to the fracture (Plasma I) and before the perfusion (Plasma II) emboli bone marrow and filtrate in the non traumatized animals

Table VII The analyses of variances Emboli vs Plasma I and II emboli vs bone marrow in the traumatized dogs A1 A7

Fractions tested	Source of variation	Sums of squares	Degrees of free dom	Mean squares	F
Emboli Plasma I	Between animals	27	6	4.5	0.005
	Between fatty acids	176485	15	11765.7	12.477[1]
	Remainder	91468	97	943.0	
	Total	267980	118		
Emboli Plasma II	Between animals	30	6	5	0.007
	Between fatty acids	184[illegible]8	15	12308.5	16.431[1]
	Remainder	[illegible]2665	9	[illegible]49.1	
	Total	[illegible]3[illegible]3	118		
Emboli Bone Marrow	Between animals	68	6	11.3	0.041
	Between fatty acids	1786	1	119.1	0.4[illegible]7
	Remainder	2704	[illegible]	[illegible]8.8	
	Total	28894	118		

Table VIII The correlations in fatty acid percentages in the triglycerides emboli vs plasma and emboli vs bone marrow (dogs A1–A7) The correlations were compared by Snedecor's F test applied to the residual variances (s^2_{yx}) * indicates that the relationship between emboli vs bone marrow is closer ($p < 0.01$) than emboli vs both plasma I and II

Dog	Emboli–Plasma I r	Emboli–Plasma I s_{yx}	Emboli–Plasma II r	Emboli–Plasma II s_{yx}	Emboli–Bone marrow r	Emboli–Bone marrow s_{yx}
A1	0.65	8.6	0.81	6.6	0.99	1.7*
A2	0.99	1.5	0.99	1.8	1.00	0.5*
A3	0.96	4.0	0.92	5.7	1.00	0.6*
A4	0.98	2.3	0.98	2.5	1.00	0.8*
A5	0.97	3.0	0.95	3.5	0.99	1.3*
A6	0.84	5.8	0.87	5.3	0.97	2.4*
A7	0.99	1.0	0.99	1.7	0.99	1.4

[1]) p 0.001

could be interpreted, that if a formation of emboli from plasma lipid occurs this should be most pronounced within 1 hour after the trauma

The method for isolation of the fat emboli by retrograde perfusion of the pulmonary vessels gives both a possibility to analyse the chemical composition of the emboli and a method for quantitating the amount of emboli fat present in the lungs

The amount of emboli washed out was sufficent for the chemical analyses but all emboli were not washed out in all animals Therefore the question was raised as to whether these variations in yield were due to the composition of the emboli or to the size Since the emboli composition in those dogs with a marked residue of emboli was similar to those in which almost all emboli were removed differences in lipid composition of the emboli cannot explain the differences in yield Furthermore the microscopic investigation did not indicate that only emboli of a certain size were removed in animals with an incomplete yield

The histological methods for the analyses of the amount of fat emboli are only semi quantitative as has been pointed out by several authors (4 51 54) Also the present method for the quantitation of emboli includes errors There were in many experiments emboli left in the lung vessels, and some emboli might have been lost via the leak through the bronchial arteries In this limited material the results do not permit any definite conclusions concerning the relationship between the two methods for quantitating the fat emboli Therefore the quantitative aspects of the present technique will be discussed in a forthcoming publication (35) and the present discussion will be limited to the qualitative emboli analyses

The small amount of cholesterol and phospholipids found in the emboli fractions could either be a part of the emboli or derived from impurities in the form of lipoproteins or blood corpuscles stuck on the filters The amount of cholesterol and phospholipids was roughly the same in both non traumatized and traumatized animals and was not correlated to the amount of triglycerides indicating that the main part of cholesterol and phospholipids was not a part of the emboli but probably derived from blood corpuscles (Se also p 5)

An accurate estimation of the triglyceride content of the emboli could be made in those experiments in which a great amount of lipid material was found on the filters i e in dogs 44–45 In these animals the emboli fraction contained approximately 98–99 per cent of triglycerides of the total lipids

There are reasons to believe that the triglycerides in different parts of the adipose tissue are rather similar (30 31) and comparisons of the fatty acid composition in subcutaneous fat and bone marrow fat in dogs performed in our laboratory with the present methods have confirmed these observations (35) The triglycerides in plasma differ from those in adipose tissue especially in the percentage of C18 1 C18 2 C20p and C22p fatty acids The striking similarity in the triglyceride fatty acid composition between the emboli and bone marrow fat and the discrepancy in composition between the emboli and plasma triglycerides indicates strongly that the bone marrow fat is the source of the fat emboli in these traumatized dogs Furthermore the plasma triglycerides did not alter their fatty acid pattern during the experiment Therefore the possibility that a plasma triglyceride fraction with the same fatty acid pattern as bone marrow fat could appear in the plasma after the trauma and act as source for the emboli seems unlikely

However one must consider that although the bone marrow is an anatomical unit the bone marrow fat physiologically is a part of the adipose tissue The conclusion from the present experiments therefore is that the emboli either are caused by mechanical liberation of bone marrow fat or by a selective accumulation in the lungs of similar triglycerides synthesized from adipose tissue fatty acids outside this tissue presumably in the liver

Concerning the genesis of post traumatic

fat embolism there are many observations reported which argue in favour of the theory that the traumatized tissue fat is the source of the fat emboli Most of these observations have been made in patients with fractures and in animals with experimentally produced fractures Marrow fat is freed after fracture and is visible in the haematoma in the fracture region As much as 30-60 ml of liquid fat has been found in haematoma around the fracture (16 57) The possibility for freed fat to enter the circulation is facilitated in fractures as the veins are attached to bone canal and therefore do not collapse (27) In many traumatic injuries an increased pressure in the fracture areas can be present and might be of importance for the embolisation It should be mentioned however that medullar nail n [illegible] Kun hner nailing) which cause elevation of the intra medullar pressure [illegible] not reported to be associated [illegible] frequency of clinical fat emb[illegible] [illegible] that an increased intramedullar pressure is of minor [illegible] suggested that th[illegible] movements m[illegible] in fat embolisation The [illegible] and Griffith [illegible] that the pr[illegible] must be higher than in[illegible] enter the blood str[illegible] some kind of pre[illegible] needed to make the freed [illegible]

Ge[illegible] a correlation between the d[illegible] fat embolism and the severity [illegible] but marked emboli sation [illegible] observed after injuries of small [illegible] The embolisation seems to be d[illegible] prevented by prior ligation of vein[illegible] the traumatized region (47) The[illegible] of course be substances of pathogene[illegible] significance for fat embolisation which are retained in the traumatized region if the draining veins are occluded Several authors have reported that pieces of bone marrow can be found in the lung vessels after fractures (9 19 21) Pulmonary fat emboli can be traced from marrow fat after staining the marrow with a dye (10 25) Fuchsig Brucke Blumel & Gottlob (20) observed that hypovolemia increased the rate of elimination of labelled fat injected into the muscles and that the labelled fat was recovered in the lungs However fat emboli are observed also in individuals without initial signs of hypovolemia or general disturbances of the circulation Hypovolemia does not increase the number of emboli in experimental animals with fractures (35 42 43)

The physiochemical theory has been stressed especially by Lehman & Moore 1927 (38) and further discussed by Johnson & Svanborg 1956 (33) by Bergentz Gelin Hallgren Pudenstam & Svanborg 1962 (6) and by Bergentz 1961 and 1968 (4 5) The reasons that these authors suggested another source of the fat emboli than the bone marrow were manyfold Fat emboli of a considerable histological frequency and sometimes combined with the clinical symptoms referred to fat embolisation have been recorded in a large number of patients without traumatic injuries to bones or soft tissues There exists a discrepancy between the extent of the traumatic injury and the frequency and magnitude of fat embolism The appearance of the fat emboli syndrome continues for a remarkably long time after the trauma The clinical symptoms referred to fat embolisation usually appear at first several hours to several days after the trauma The emboli occur in capillary systems distal to the lungs such as in the brain and in the kidney vessel this objection may however be less valid as Prinzmetal Ornitz Simkin & Bergman 1948 (48) and Tobin 1952 (56) have found that some pulmonary capillaries have wider diameter than for example the brain vessels Emboli may therefore pass the pulmonary capillaries and be caught in other regions

One fact that does not fit in well with the mechanical theory is that fat embolisation causing death has been observed for example when a lean foot had been traumatized and only small bones in the foot had been fractured (37) According to experiments in rabbits

(3) 0.15 g/kg body weight is needed to produce a histological gross fat embolism in the lungs, which indicate that about 10 g of fat should be needed in adult humans and about 3 g in a dog weighing 20 kg.

Another observation favouring the physio-chemical theory was made by Kronke 1956 (36) who found the amount of fat to be of the same order of magnitude in the fractured and in the non-fractured tibia in one case of fatal fat embolisation. Furthermore, Swank & Dugger 1954 (54) and Bergentz 1961 (4) claimed that hyperlipemia increased the number of pulmonary fat emboli in experimental animals. It should however be mentioned that in most experiments of Bergentz intravenous lipid emulsions were used to produce hyperlipemia. Swank, Glinsman & Sloop 1960 (55) stated that fat emboli could be produced in rabbits by feeding high fat meals. The observations of Swank *et al* (54, 55) and of Bergentz (4) could not however be confirmed by Peltier 1955 (46) or by Brody, Meadows & Zarafonetis 1962 (7). The recent observations that fat embolisation may occur during cardio-pulmonary bypass (37, 41, 45) presumably through a denaturation of the protein part of the lipoproteins strongly favours the opinion that the blood lipids under certain circumstances can be the source of fat emboli (45).

Traumatic injuries as well as many other conditions known to cause fat embolisation also influence the coagulability of the blood and the capillary circulation (4, 5, 23). Several investigations have demonstrated that traumatic injuries produce aggregation of platelets and other blood corpuscles, so called white emboli (4, 40). According to Bergentz *et al* (6) such emboli were detected on the top of the red cell column after the centrifugation of the blood and included droplets of triglycerides. These droplets probably have a fatty composition similar to that of plasma triglycerides (24). There was also a higher amount of both the white emboli layer on the red cell column and of the fat content in the whole lung tissue in animals traumatized in a post-alimentary phase than in those traumatized in a post-absorptive phase (6). These observations were also taken as evidence in favour of the concept that the blood lipids contribute to fat emboli. O'Driscol and Powell (44) reported that when the plasma lipids were lowered by treatment with clofibrate (Atromidin©) the incidence of fat embolism decreased. However, as earlier mentioned Peltier (46) and Brody *et al* (7) did not observe that hyperlipemia influenced the frequency of fat emboli after traumatic injury. Bergentz (4) postulated that the increased coagulability after trauma resulted in an aggregation of chylomicrons resulting in the formation of fat emboli. It should be pointed out, however, that a trauma which causes only moderate alterations in the coagulation system might cause gross fat embolisation, while injection of high amounts of thromboplastic substances causing drastic alterations in the coagulability of the blood has been needed in order to produce a significant degree of fat embolism (2, 4).

Various observations of fat emboli in other forms of so called non-traumatic conditions are referred to as arguments favouring the blood lipids as source of the emboli. Grondahl (26) reported that necrotizing pancreatitis may result in gross fat embolisation. The necrotizing fat tissue may, however, act as the source of the emboli. Fat emboli have also been observed in a number of septic infections, intoxications and diabetes (for a review see Lehman & Moore (38) and Sevitt (51)). In several of these conditions tissue necrosis in fat rich organs, as for example in fatty livers, may be present.

In 1956 Bruyans & Fiseman (8) proposed that the increased sympathetic activity produced by a trauma was in some way responsible for the embolisation. Later studies have shown (for a review see Carlson *et al* (12)) that increased sympathetic activity increases the mobilisation rate of free fatty acids (FFA) from the adipose tissue and causes accumulation of triglycerides in the liver (13, 18), in some muscle fibers and in the lung parenchyma

(13) and also increases the plasma triglycerides after 12 hours of norepinephrine infusion Excessive FFA mobilisation caused by nor epinephrine infusion for 24 hours caused *per se* no significant fat embolisation (13) However, Liljedahl & Westermark (39) have put forward a modification of the physiochemical theory based both on the blood lipid changes during stress situations and on the changes produced by the trauma on the blood coagulation system Their theory claims that fat embolisation is the result of a traumatic stress induced intravas cular coagulation and disturbances in fat meta bolism

Kronke (36) found a rise in serum lipo protein lipase activity after fractures and postulated that this results in increased mobilisa tion of adipose tissue triglycerides which appear in the fat emboli However the sum of observations do not indicate that heparin administration known to increase the lipo protein lipase activity has any harmful effects in patients with fat embolism (1 15 17 39)

Concerning the genesis of post traumatic fat embolism, the previous investigations can be summarized as follows

There is much evidence that fat from traumatized fat rich tissues can enter the blood stream and form fat emboli There is also much evidence that fat emboli can be formed through other mechanisms as for example from trau matized blood lipoproteins in cardio pulmonary bypass This latter conclusion should, however not be interpreted as a proof that the bone marrow is not the source of the emboli after skeletal trauma The conclusion should rather be that more than one possibility exists for the origin of fat emboli The striking simi larity in the composition of the emboli fat and bone marrow fat in the present study indicates strongly that the bone marrow fat is the source of the emboli in these trau matized animals The present animals were studied under fasting conditions Therefore the present results do not exclude the possibility that the larger lipoproteins appearing in a post alimentary phase may contribute to the fat in the lung vessels This possibility has been studied separately and will be reported on in separate publications Furthermore the present results do not contradict the view that the traumatic injury releases adipose tissue fatty acids, which then are resynthesized into triglycerides which then appear in the lung vessels This possibility has also been further investigated and is reported on in separate articles

ACKNOWLEDGEMENT

The study was supported by the Swedish Medical Research Council, Forsvarsmedicinska sektionen (Projekt 40X 998)

REFERENCES

1 Adler F & Peltier L F The effect of sublingual potassium Heparin (Clarin) on the serum lipase activity of patients following fractures J Trauma 4 390 1964

2 Adkins R B & Foster J H Experimental study of the genesis of fat embolism Ann Surg 156 515 1962

3 Armin J & Grant R T Observations on gross pulmonary fat embolism in man and the rabbit Clin Sci 10 441 1951

4 Bergentz S E Studies on the genesis of post traumatic fat emboli m Acta chir scand Suppl 2 2 1961

5 Bergentz S E Fat embolism Progr Surg 6 85 Karger Basel/New York 1968

6 Bergentz S E Celin L E Hallgren B Rudenstam C M & Svanborg A The influence of trauma on the lipids in blood and lung tissue Acta chir scand 124 377 1962

7 Brody G L Meadows Th R & Zarafonetis Ch J D Hyperlipemia and fat embolism Amer J med Sci 247 682 1964

8 Bruyans W A & Eiseman B The incidence of fat globulemia following soft tissue and ortopedic operations Surg Forum 6 28 1955

9 Buchner Ch Traumatische Knochenmarksembolie der Lungen zugleich ein Beitrag zur Pathogenese der Fettembolie Dtsch med Wschr 89 1390 1964

10 Busch F Über Fettembolie Virschows Arch path Anat 35 321 1866 (cited by Sevitt)

11 Carlson L A Determination of serum triglycerides J Atheroscler Res 3 334 1963

12 Carlson L A Boberg J & Hogstedt B Some physiological and clinical implications of lipid mobilization from adipose tissue In Handbook of Physiology Section 5 Chapter 63 Waverly Press Baltimore USA 1965

13 Carlson L A Liljedahl S O & Wirsén C Blood and tissue changes in the dog during and after excessive free fatty acid mobilization Acta med scand 178 81 1965

14 Carroll K K Dietary fat and the fatty acid composition of tissue lipids J Amer oil chem Soc 42 516 1965

15 Derian P S Fat embolization—current status J Trauma 5 80 1965

16 Donnell J M Observations of clinical fat embolism Canad med Ass J 86 1060 1962

17 Evarts Ch M Diagnosis and treatment of fat embolism J Amer med Ass 194 899 1965

18 Feigelson E B Pfaff W W Karmen A & Steinberg D The role of plasma free fatty acids in development of fatty liver J clin Invest 40 2171 1961

19 Fisher J H Bone Marrow embolism AMA Arch Path 52 315 1951

20 Fuchsig P Brucke P Blumel G & Gottlob R A new clinical and experimental concept on fat embolism New Engl J Med 276 1192 1967

21 Garvey J W & Zak F G Pulmonary bone marrow emboli in patients receiving external cardiac massage J Amer med Ass 187 159 1964

22 Gauss H The pathology of fat embolism Arch Surg 9 593 1924

23 Gelin L E Studies in anemia of injury Acta chir scand Suppl 210 1956

24 Gelin L E Hallgren B Kerstell J Rudenstam C M & Svanborg A The lipid composition of lung plasma red corpuscle column and bone marrow in pulmonary fat embolism Acta chir scand 133 107 1967

25 Glas W W Grekin T D Davis H L & Musselman M M An experimental study of the etiology of fat embolism Amer J Surg 91 471 1956

26 Grondahl N B Untersuchungen uber Fettembolie Dtsch Z Chir 111 56 1911

27 Gustafson A & Kerstell J Unpublished

28 Hallgren B Kerstell J Rudenstam C M & Svanborg A A method for the isolation and chemical analysis of pulmonary fat emboli Acta chir scand 132 613 1966

29 Hallgren B Stenhagen S Svanborg A & Svennerholm L Gas chromatographic analysis of the fatty acid composition of the plasma lipids in normal and diabetic subjects J clin Invest 39 1424 1960

30 Hirsch J Composition of adipose tissue In Adipose tissue as an Organ Ed L W Kinsell Ch C Thomas publisher Illinois USA 1962

31 Imaichi K Fukada J I Oyama K & Mukawa A Effect of sample site on fatty acid composition of adipose tissue J Biochem 58 463 1965

32 Johnson S R Rieger A & Svanborg A Fat embolism Acta chir scand 110 389 1956

33 Johnson S R & Svanborg A Investigations with regard to the pathogenesis of so called Fat embolism Ann Surg 144 145 1956

34 Kerstell J The incorporation of labelled chylomicrons into fat emboli in post alimentary dog Acta med scand Suppl 499 33 1969

35 Kerstell J Studies on the pathogenesis of post traumatic fat embolism Thesis Elanders Boktryckeri Goteborg Sweden 1969

36 Kronke E Die Bedeutung der Lipase in der Pathogenese der traumatischen Fett Embolie Langenbecks Arch klin Chir 283 466 1956

37 Lee W H Jr Krumhaar D Fonkalsrud F W Schjeide O A & Maloney J V Denaturation of plasma proteins as a cause of morbidity and death after intracardiac operations Surgery 50 29 1961

38 Lehman E P & Moore R M Fat embolism Arch Surg 14 621 1927

39 Liljedahl S O & Westermark L Aetiology and treatment of fat embolism Acta anaesth scand 11 177 1967

40 Lutz B R Fulton G P & Akers R P White tromboembolism in the hamster cheek pouch after trauma infection and neoplasia Circulation 3 339 1951

41 Miller J A Fonkalsrud E W Latta H L & Maloney J V Jr Fat embolism associated with extracorporeal circulation and blood transfusion Surgery 51 448 1962

42 Morton K S & Gurjar M P Hemorrhagic shock and fat embolism A clinical and experimental study J Trauma 4 687 1964

43 Morton K S & Kendall M J Fat embolism its production and source of fat Canad J Surg 8 214 1965

44 O Driscoll M & Powell F J Injury serum lipids fat embolism and clofibrate Brit med J 4 149 1967

45 Owens G Adams J E & Scott H W Jr Embolic fat as a measure of adequacy of various oxygenators J appl Physiol 15 999 1960

46 Peltier L F Fat embolism The failure of lipemia to potentiate the degree of fat embolism accompanying fractures of the femur in rabbits Surgery 38 720 1955

47 Peltier L F Fat embolism The prophylactic value of a tourniquet J Bone Jt Surg 38A 835 1956

48 Prinzmetal M Ornitz E M Jr Simkin B & Bergman H C Arterio venous anastomoses in liver spleen and lungs Amer J Physiol 152 48 1948
49 Romeis B Mikroskopische Technik 15 Auflage Oldenburg Munchen 1948
50 de Ruiter H Traumatic fat embolism Thesis Drukkerij Noord Holland N V Hoorn 1966
51 Sevitt S Fat embolism Butterworth & Co London 1962
52 Snedecor G W & Cochran W G Statistical methods 6th ed The Iowa state University Press Ames Iowa 1967
53 Svanborg A & Svennerholm L Plasma total lipid cholesterol triglycerides phospholipids and free fatty acids in a healthy scandinavian population Acta med scand 169 43 1961
54 Swank R L & Dugger G S Fat embolism Surg Gynec Obstet 98 641 1954
55 Swank R L Glinsman W A Sloop P The production of fat embolism in rabbits by feeding high fat meals Surg Gynec Obstet 110 9 1960
56 Tobin Ch E The bronchial arteries and their connections with other vessels in the human lung Surg Gynec Obstet 95 741 1952
57 Vance B M The significance of fat embolism Arch Surg 23 426 1931
58 Wehner C Morgenstern C & Zeumer, G Das Verhalten des intramedullaren Drucks bei Markbohrung und nagelung Zbl Chir 91 209 1966
59 Young J S & Griffith H D The dynamics of parenchymatous embolism in relation to the dissemination of malignant tumours J Path Bact 62 293 1950
60 Zenker F A Beitrage zur normalen und pathologischen Anatomie der Lunge p 31 Dresden J Braunsdorf 1862 (cited by Sevitt)
61 Zlatkis A Zak B & Boyle A J A new method for the direct determination of serum cholesterol J Lab clin Med 41 486 1953

FROM THE DEPARTMENT OF GERIATRICS II, VASA SJUKHUS, THE DEPARTMENT OF SURGERY I, THE DEPARTMENT OF MEDICINE I AND THE DEPARTMENT OF MEDICINE II, SAHLGRENSKA SJUKHUSET, UNIVERSITY OF GÖTEBORG, GÖTEBORG, SWEDEN

II. SERUM LIPOPROTEIN PATTERN IN FAT EMBOLISM IN THE DOG

BY

A. GUSTAFSON and J. KERSTELL

Abstract. Quantitative and qualitative serum lipoprotein studies in the dog during fat embolisation did not reveal any major changes within the lipoprotein distribution indicating that none of the lipoproteins participate as a source or transport vehicle for fat emboli material. An expected major participation by the HDL (high density lipoproteins) or α-LP (α-lipoproteins) in the transport of fat emboli material in the dog cannot be confirmed by the present study.

It is now a well-established fact that all lipids present in the blood appear under normal conditions bound to one or several protein moieties. Lipoproteins varying in density (*D*) from 0.9 g/ml to 1.2 g/ml are present in the serum. In human serum D 1.063 g/ml separates low density (LDL) from high density lipoproteins (HDL). The least dense lipoproteins D<0.95 g/ml characterized by large size and a high content of triglycerides are also called particulate fat. The biggest lipoproteins are the chylomicrons with a protein content of less than 1% (2). In human chylomicrons this protein is predominantly protein A also characteristic for the HDL (2). The HDL and its protein moiety, protein A, in man appears to be a protein moiety with high affinity for triglycerides. This affinity can also be shown in *in vitro* experiments (8).

The aim of the present investigation was to study by preparative ultracentrifugation and paper electrophoresis whether emboli material could appear as particulate fat and secondly to investigate the serum lipoprotein pattern before and during fat embolisation. It was hypothesized that the fat emboli material was transported partly as lipoproteins complexed with HDL or protein A. For this hypothesis to be correct one would expect a consumption of HDL during fat embolisation.

MATERIAL AND METHODS

Three post-absorptive dogs were traumatized as described previously (5) and the fat emboli subsequently isolated.

Three to four hours after the fracture the pulmonary circulation was isolated and perfused in the retrograde direction with isotonic citrate solution. The perfusate was then passed through a filter with a pore size of 28 μ, centrifuged at 1400 g for 20 min. at 5 C and finally passed through a new filter with pore size of 28 μ.

The main part of the emboli in the perfusate was caught in the filters.

The histological sections were stained with osmic acid and the emboli counted in 6–9 cm² of lung tissue from one non-perfused lobe from each dog.

In dog X1 and dog X2 the serum lipoprotein patterns were studied before, one hour and three hours after the injury and in dog X3 lipoprotein electrophoresis was performed on serum samples obtained at 15 minutes intervals after the trauma.

Blood samples were obtained from a brachial artery. The blood was centrifuged at 2000 g for ten minutes to remove corpuscular elements. Fresh serum samples were used for the subsequent lipoprotein analysis. The LDL

were separated from the HDL by preparative ultracentrifugation at D 1 063 g/ml at 105 000 g for 22 hours The density was obtained by the addition of NaCl and was checked by hydrometer The centrifuge tubes were cut by a tube slicer and the LDL and HDL were recovered from supra and infranatants respectively The isolated lipoproteins were extracted and total cholesterol phospholipids and triglycerides were determined as described earlier (5)

Lipoprotein separation was also performed by column chromatography on hydroxyl apatite according to Hjerten (4) Ten ml of serum was applied to a 100 ml hydroxyl apatite column and eluated with *0 25 M and 0 65 M phosphate* buffer From experience with human serum it is known that 0 25 M phosphate buffer elutes α lipoproteins (α LP) together with serum proteins and that the 0 65 M fractions contains β lipoproteins (β LP) except for particulate fat of $D < 0.95$ g/ml (1 3) The eluation volumes were lyophilized and extracted for lipids according to the method described earlier (5) Total cholesterol phospholipids and triglycerides were determined in the lipid extracts (5)

Lipoprotein paper electrophoresis was performed according to Lees & Hatch (7) employing veronal buffer containing 1% albumin The *paper strips were stained with a lipid stain* Fett Rot 7 B (Ciba) for two hours and the strips subsequently washed in running tap water overnight No attempts to quantitate the lipoprotein bands were made Agarose gel electrophoresis was performed according to Williams & Grabar (10) utilizing a modified discontinuous buffer system (6) Two to 6 separate rectangular basins were perforated and used as filling reservoirs Amido Schwartz 10 B (Bayer) and Oil Red 0 (Spinco) were employed to stain protein and lipid respectively

RESULTS

All dogs tolerated the trauma well In dog X3 only transverse fractures with very smooth margins were obtained and almost no fracture hematomas were observed

Table I Number of emboli/cm² in the control lobes and total amount of triglycerides in emboli material washed out from the pulmonary circulation in the three dogs TG = triglycerides

	Number of *emboli/cm²* lung tissue	Total amount TG *in emboli* (mg)
Dog X1	6	31
Dog X2	5	63
Dog X3	1	0 4

The number of emboli per cm² of lung tissue in the non perfused lobes and the amount *of triglycerides in the emboli fractions washed* out from the pulmonary circulation in each of the three dogs are given in Table I In dog X3 only a minor amount of emboli triglycerides was washed out from the pulmonary vessels The lipid contents expressed as mg per 100 ml of serum in the two major lipoprotein fractions obtained by preparative ultracentrifugation and column chromatography are given for dog X1 in Table II and for dog X2 in Table III The lipoprotein lipid distribution is given for serum obtained before one hour and three hours after the injury In dog X1 31 mg of triglycerides was washed out of the pulmonary *circulation No significant change in lipoprotein* distribution was recorded during the fat embolisation A stable lipoprotein composition was found with both isolation methods in dog X1 Dog X2 with 63 mg of triglycerides obtained as fat emboli material from the pulmonary circulation showed a moderate decrease in the HDL three hours after the trauma

From the visual evaluation of the serum lipoprotein electrophoresis in dog X3 every fifteen minutes up to three hours after the injury no obvious change in lipoprotein distribution could be recorded (Fig 1) When the two *methods for lipoprotein separation* were compared differences in the lipid contents of LDL and β LP on one hand and between HDL and α LP on the other, were observed The combined LDL and HDL by the ultra

Table II Dog N1 Lipoprotein composition before the trauma and one and three hours after Fractions isolated by column chromatography and preparative ultracentrifugation at D 1 063 g/ml Data expressed as mg per 100 ml serum PL = phospholipid TC = total cholesterol TG = triglycerides

Time	Column chromatography						Preparative ultracentrifugation					
	β LP (0 65 M)			α LP (0 25 M)			L D L (D<1 063)			H D L (D>1 063)		
	PL	TC	TG	PL	TC	TG	PL	TC	TG	PL	TC	TG
Before	54	48	41	268	134	2	30	28	42	274	172	2
One hour afte	51	46	42	285	149	3	36	30	42	308	179	2
Three hours after	51	43	44	260	139	3	31	27	40	296	170	5

Table III Dog N2 Lipoprotein composition before the trauma and one and three hours after Fractions isolated by column chromatography and preparative ultracentrifugation at D 1 063 g/ml Data expressed as mg per 100 ml serum PL = phospholipid TC = total cholesterol TG = triglycerides

Time	Column chromatography						Preparative ultracentrifugation					
	β LP (0 65 M)			α LP (0 25 M)			L D L (D<1 063)			H D L (D>1 063)		
	PL	TC	TG	PL	TC	TG	PL	TC	TG	PL	TC	TG
Before	57	49	27	343	171	2	24	26	22	338	192	4
One hour after	56	46	24	321	171	3	26	27	24	337	197	3
Three hours after	43	37	23	312	163	3	25	23	25	300	173	5

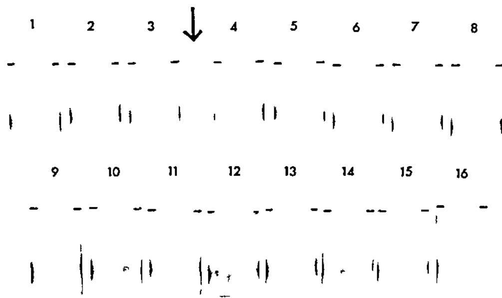


Fig 1 The electrophoretic distribution of the serum lipoproteins during fat embolisation in dog N3 The samples were obtained every 15 min The arrow indicates the fracture

FROM THE DEPARTMENT OF GERIATRICS II, VASA SJUKHUS, THE DEPARTMENT OF SURGERY I, THE DEPARTMENT OF MEDICINE II, AND THE CENTRAL LABORATORY, SAHLGRENSKA SJUKHUSET, UNIVERSITY OF GÖTEBORG, GÖTEBORG, SWEDEN

III. THE CHEMICAL COMPOSITION OF THE FAT EMBOLI IN THE POST-ALIMENTARY DOG

BY

B. HALLGREN, J. KERSTELL, C. M. RUDENSTAM & A. SVANBORG

Abstract. The composition of the pulmonary fat emboli, plasma and bone marrow triglycerides were compared in the traumatized dog in the post-alimentary state. The animals had been fed triglycerides with a fatty acid pattern different from both post-absorptive plasma and bone marrow triglycerides. This fat intake caused the appearance of chylomicrons with a triglyceride fatty acid pattern similar to that of the ingested fat. These alterations in the plasma triglycerides were not reflected in the emboli and bone marrow triglyceride composition. The results therefore indicate that there was no significant contribution of the chylomicrons to the fat emboli.

In previous investigations the chemical composition of pulmonary fat emboli was analysed in traumatized fasting dogs. A similarity was found between the fatty acid composition of bone marrow and the emboli triglycerides in these dogs (5, 7). These observations indicated that fat emboli developing during a nutritional state with a low amount of triglyceride-rich large lipoproteins originate from and consist almost entirely of bone marrow triglycerides.

Several investigators have reported that the frequency of fat emboli is influenced by the nutritional state (1, 9, 10). The question as to whether hyperlipemic episodes induced by, for example, dietary fat intake augment the fat embolisation has both theoretical and therapeutic significance. The larger triglyceride-rich chylomicrons present after fat intake would theoretically more likely contribute to fat embolisation or adhere to fat particles from other origin. In the present study, in which the previously described methods (5, 7) for the isolation and chemical analyses of pulmonary fat emboli were used, the fatty acid composition of fat emboli, plasma and bone marrow triglycerides were compared in dogs given coconut butter or synthetic tristearate, which influenced the composition of the triglycerides in plasma but did not change the composition of the bone marrow fat.

MATERIAL AND METHODS

Ten dogs weighing 17–35 kg were used. They had been fasting over night. However, in dog B1 a very high initial triglyceride level was observed which may indicate a delayed resorption of the last meal. Dogs B1–B4 and B C1 received 125 g of coconut butter and dogs C1–C3, C C1 and C C2 received 50 g of a synthetic tristearate preparation (Tristearin Fluka) mixed with ordinary food. Three hours later femoral fractures were produced in dogs B1–B4 and C1–C3. Dogs BC1, CC1 and CC2 were used as non-traumatized controls. The experimental procedures, the isolation of the emboli, the chemical and histological analyses were performed as described previously (7).

Three to four hours after the fracture the pulmonary circulation was isolated and perfused in the retrograde direction with isotonic citrate solution. The perfusate was then passed through a filter with a pore size of 28 μ, centrifuged at 1400 g for 20 min. at 5°C and finally passed

through a new filter with a pore size of 28μ The main part of the emboli in the perfusate was caught in the filters

The efficiency of the perfusions was analysed by comparison of the number of emboli in a non perfused lobe with that in the perfused lobes The histological sections were stained with osmic acid and the emboli counted in 6–9 cm² of lung tissue from each lobe

Arterial plasma samples were taken before the administration of food (Plasma 0) immediately before the fracture or corresponding time (Plasma I) and before the isolation of the emboli (Plasma II) In dog B3 and C1 serum lipoprotein classes were separated by an ultracentrifugation technique (4) The lipids in these fractions were analysed in the same way as the lipids in the whole plasma fraction

The lipids in the filter fractions and the perfusate passing both filters were called emboli and filtrate respectively and bone marrow specimens bone marrow The statistical analyses were performed as given previously (7)

RESULTS

The administration of the fats caused a pronounced steatorrhea in all dogs receiving tristearate Steatorrhea was also noted in the dogs fed coconut butter The number of fat emboli/cm² of lung tissue is shown in Table I In the dogs receiving coconut butter dog B3

Table I Traumatized dogs The number of emboli in the nonperfused control lobes and the mean number remaining in the perfused lobes Dogs B1 B4 had received coconut butter and dogs C1–C3 a synthetic tristearate preparation

Dog	Control lobe emboli cm²	Perfused lobes mean emboli/cm²
B1	1	0
B	1 5	0 6
B3	a few	a few
B4	363	16
C1	14	6
C°	1	0
C3	1	0 1

Table II Triglycerides (TG) cholesterol (Chol) and phospholipids (Pl p) in the plasma (mg 100 ml) before the administration of coconut butter or tristearate (Plasma 0) before the fracture (Plasma I) and before the perfusion (Plasma II) The lipid values for the emboli and filtrate fractions are given as mg in the total fraction The dogs denoted B were traumatized and had received coconut butter dog BC was not traumatized Dogs denoted C were traumatized and had received a synthetic tristearate preparation and dogs CC were not traumatized

	Plasma 0			Plasma I			Plasma II			Emboli			Filtrate			Filtrate amount plasma TG calculated from	
1	I	Chol	Pl p	TG	Chol	Pl p	TG	Chol	Pl p	TG	Chol	Pl p	TG	Chol	Pl p	Hb	Pl p
I	4	113	86	91	119	90	58	161	°51	31		1	1°	5	140	30	3
L	8	111	°63	4	1 4	83	19	115	63	14	0 6	0 9	3	98	198	18	14
B3	1	16	335	54	144	°68	117	1 8	360	9	0 5	1	16	71	13	39	43
B4		138	301		130	°6°	1	116	°18	1 55	3	0 3	111	14		3	
B (	44	307	464	34	98	493	5	°49	450	(	1		9	91	16		9
C1	°	4	44	1	°44	4 5	9	3	441	85		1	88	93	150	34	31
C	61	155	346	168	149	345	35	166	356	1			19		130	1	13
C3	64	303	43	81	46	368	66	51	3 6	9		4	6	16	°4	39	4°
C C1	36	104	18		1 3	180	8	100	163	0 6	1	°	6	4°	60	5	8
C C	3	1 9	°17	8	1 8	° 6	0	107	°40	1			5		46	13	10

Table III The fatty acid composition of the triglycerides in plasma before the administration of fat (PO) before the fracture (PI) and plasma before the perfusion of the pulmonary circulation (PII) fat emboli fraction (F) bone marrow (BM) and filtrate (I) in all dogs The emboli fraction was not analysed in the non traumatized dogs BCI and CCI–CC2 The I O fraction was not analysed in dogs B4 and C3

	B1						B2						B3						B4					BC1				
	PO	II	III	I	BM	I	PO	PI	III	E	BM	F	PO	PI	PII	L	BM	I	PI	PII	I	BM	F	IO	II	PII	BM	F
12	04	48	102	02	04	43	04	157	78	07	05	02	05	154	274	07	05	82	10	0	05	04	0	05	119	107	09	93
14	38	[illegible]	[illegible]	24	30	65	17	92	78	26	30	24	22	100	171	40	34	113	23	15	47	38	14	37	83	103	25	80
15	11	[illegible]	03	[illegible]	03	10	06	11	03	06	08	05	08	07	07	05	05	05	10	07	10	11	07	04	0	03	06	06
160	249	[illegible]	181	211	196	250	234	218	231	[illegible]	240	268	249	207	174	251	251	238	177	178	195	217	187	253	198	208	253	227
161	48	37	30	53	41	40	45	50	41	48	41	43	30	24	19	32	29	29	41	35	76	65	50	24	25	16	31	16
17	[illegible]	18	09	11	12	20	15	14	07	20	14	13	15	09	09	10	12	09	21	23	21	22	23	07	15	05	08	10
180	157	131	114	90	102	124	[illegible]	63	61	90	113	85	67	82	54	75	70	73	77	89	73	84	83	89	96	105	87	80
181	406	349	307	500	572	348	434	278	315	449	453	399	367	291	189	443	470	326	397	354	484	493	513	378	284	204	475	277
182	31	34	41	77	74	35	74	43	84	72	43	76	117	72	41	74	77	62	93	84	56	21	55	111	95	91	76	40
19	02	02	01	03	01	04	04	03	02	03	08	03	03	02	02	02	04	03	08	11	03	07	07	02	01	0	02	02
200	06	07	03	05	[illegible]	06	03	04	03	04	03	10	03	03	03	05	04	03	03	04	02	02	03	03	02	03	03	03
201	04	08	04	13	10	10	13	13	08	04	18	13	13	09	07	12	13	09	09	15	14	13	10	07	05	06	12	0
20p	10	14	18	[illegible]	01	26	51	21	60	23	05	27	61	24	19	04	06	13	94	134	05	25	17	47	57	48	05	05
21	0	01	02	0	0	04	06	06	01	04	02	07	0	0	0	0	0	0	03	07	02	0	0	0	0	0	0	0
220	0	02	02	01	0	0	0	0	0	04	0	0	19	10	19	34	15	26	03	13	05	03	11	21	10	23	05	131
221	02	08	06	03	01	07	04	17	15	16	09	18	03	06	06	08	03	05	0	03	04	02	05	02	04	04	02	0
22p	04	06	08	01	01	09	17	14	15	09	07	08	18	10	06	01	02	04	31	28	01	02	03	11	20	14	02	30

	C1						C2						C3					CC1					CC2				
	PO	PI	PII	E	BM	F	PO	PI	PII	E	BM	F	PI	PII	E	BM	F	PO	PI	III	BM	F	PO	II	PII	BM	F
12	03	04	05	0	03	07	0	03	23	03	0	16	02	0	08	04	02	0	03	04	0	09	02	05	31	12	14
14	21	35	31	30	34	33	21	87	60	22	22	45	14	12	33	24	16	11	35	16	21	28	21	48	52	43	43
15	11	08	08	02	0	0	06	08	07	04	01	06	06	06	08	03	05	0	04	09	01	08	07	07	04	06	07
160	231	[illegible]	252	253	255	255	232	238	226	238	254	231	238	251	242	255	243	262	249	234	258	230	249	238	191	232	201
161	19	13	16	32	29	30	31	16	18	34	30	20	15	15	26	22	13	13	09	14	25	19	17	10	13	29	15
17	24	15	16	07	05	17	10	09	12	04	05	13	11	13	11	06	11	18	11	21	05	28	15	13	13	07	15
180	86	149	139	71	79	75	85	183	178	77	101	145	122	123	92	109	151	123	169	148	95	127	92	226	280	71	218
181	364	380	377	479	473	470	385	293	291	491	442	379	377	378	461	456	387	380	370	352	462	341	285	268	208	416	[illegible]
182	103	69	[illegible]	73	79	77	92	42	66	66	62	56	119	92	71	68	80	98	72	85	78	69	118	61	57	101	71
19	04	01	03	03	02	02	02	02	07	04	02	03	02	03	03	03	03	02	03	03	02	03	0	02	02	03	04
200	04	03	04	04	04	03	05	07	04	04	06	06	04	08	04	05	08	06	06	04	10	06	06	08	05	05	10
201	11	10	11	12	11	09	13	38	37	27	23	27	13	13	22	22	15	13	13	14	22	13	15	28	30	44	35
20p	68	30	36	26	14	10	56	12	22	05	02	15	45	40	03	03	35	32	21	38	03	23	93	[illegible]	31	04	24
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[illegible]0	20	16	13	10	08	10	02	13	10	16	27	16	16	19	11	14	19	25	20	34	13	79	52	21	15	08	35
[illegible]1	02	0	02	01	02	0	37	37	29	06	11	15	0	01	08	05	01	03	02	0	03	11	0	19	36	14	20
[illegible]	29	13	14	01	02	02	22	14	15	05	13	09	17	17	03	01	12	14	15	25	04	06	28	19	23	05	20

Table IV The amount (mg/100 ml) of cholesterol (Chol) phospholipids (Plip) and triglycerides (TG) in the various serum lipoprotein fractions and in plasma immediately before the fracture in dogs B3 and C1 The fatty acid composition of the triglycerides of the chylomicrons VLDL plasma and of the administered fats

VLDL = Very low density Lipoproteins LDL = Low density Lipoproteins HDL = High density Lipoproteins

	Chylo microns (S_f > 400)	VLDL (S_f 20 400)	LDL (S_f 0–20)	HDL	Plasma
Dog B3					
Chol	4	2	38	76	144
Plip	7	3	77	146	268
TG	36	6	8	6	54
Dog C1					
Chol	7	1	67	1[illegible]2	244
Plip	9	4	83	200	4[illegible]5
TG	56	12	36	[illegible]	1[illegible]

Table V The correlations in the fatty acid percentages in the triglycerides emboli vs plasma and emboli vs bone marrow The correlations were compared by Snedecor s F test applied to the residual variances (s^2_{yx}) * indicates that the relationship between emboli vs bone marrow is closer ($p < 0 01$) than emboli vs both plasma I and II

	Emboli – Plasma 0		Emboli – Plasma I		Emboli – Plasma II		Emboli – Bone marrow	
Dog	r	s_{yx}	r	s_{yx}	r	s_{yx}	r	s_{yx}
B1	0 97	3 2	0 95	4 [illegible]	0 84	7 1	1 00	0 7*
B2	1 00	1 1	0 86	6 0	0 95	3 8	0 99	1 2*
B3	0 98	2 5	0 88	5 7	0 54	10 1	1 00	0 5*
B4			0 97	3 2	0 93	4 6	0 99	1 2*
C1	0 98	2 3	0 97	2 9	0 98	2 7	1 00	0 5*
C2	0 98	2 5	0 90	5 7	0 91	[illegible] 2	0 99	1 2*
C3			0 98	2 6	0 98	2 4	1 00	0 5*

Table IV (cont)

TG fatty acids per cent	Dog B3 Chylo microns (S_f > 400)	Dog B3 VLDL (S_f 20–400)	Dog B3 Plasma	Dog B3 Coco nut butter	Dog C1 Chylo microns (S_f > 400)	Dog C1 VLDL (S_f 20–400)	Dog C1 Plasma	Dog C1 Tri stearat
12	14 5	1 6	15 4	34 6	0 4	0	0 4	0
14	13 9	4 4	10 0	20 8	[illegible] 6	2 1	3 5	0 3
15	0 4	0 7	0 7	0 3	0 7	0 8	0 8	0 2
16 0	22 7	24 7	20 7	17 6	27 1	2[illegible] 7	25 5	11 2
16 1	1 2	3 2	2 4		1 7	1 5	1 3	0
17	0 7	2 3	0 9	0 5	1 8	1 8	1 5	2 9
18 0	9 1	10 6	8 2	16 7	20 1	17 8	14 9	70 0
18 1	24 5	32 6	28 1		34 6	32 5	38 0	
18 2	4 2	7 7	7 2		6 0	7 8	6 9	
19	0 2	0 2	0 2	0 7	0 1	0 3	0 1	1 4
20 0	0 5	0 7	0 3	1 0	0 3	0 8	0 3	3 0
20 1	1 0	0 7	0 9	0 3	0 7	1 0	1 0	0 5
20*p*	4 8	1 8	2 4		2 2	2 1	3 0	
21	0	0	0	0 8	0	0	0	1 7
22 0	1 1	7 6	1 0	3 4	1 4	4 4	1 6	5 [illegible]
22 1	0 [illegible]	0 6	0 6	0 6	0 1	0 4	0	0 3
22*p*	0 [illegible]	0 6	1 0		0 2	1 0	1 3	0

had almost no emboli and dog B4 showed massive fat embolism. In the control animals emboli were seen only occasionally.

The amount of triglycerides, cholesterol and phospholipids in plasma, emboli and filtrate fractions is given in Table II. The plasma triglyceride fatty acid composition (Table III) was altered towards the triglycerides administered (Table IV) in all dogs except for dog B4. These alterations were caused by the appearance of chylomicrons ($S_f > 400$) containing a large percentage of the fatty acids which were predominantly those of the administered fat (Table IV). The bone marrow triglyceride fatty acid patterns were similar to those of the fasting dogs in the previous papers (5, 7) and had obviously not altered after the fat intake. The emboli triglycerides were very similar to those of the bone marrow and differed from those of plasma. The results of the triglyceride fatty acid determinations in the various fractions are given in Table III and figs 1–2.

In Table V the statistical evaluation of the similarity between emboli and plasma and between emboli and bone marrow fractions is given. This shows a closer similarity between emboli and bone marrow triglycerides than between emboli and plasma triglycerides.

DISCUSSION

The administration of coconut butter or of dietary fat rich in tristearate altered the plasma triglyceride fatty acid composition obviously due to the appearance of chylomicrons with a triglyceride fatty acid pattern similar to that of the ingested food. In this investigation the different fats were administered together with the ordinary food. Because of this, the chylomicrons did not have precisely the same composition of triglycerides as the coconut butter or tristearate. The present observations agree with earlier findings that plasma triglycerides rapidly alter their fatty acid composition after dietary fat intake, while the adipose tissue triglycerides are altered only after several weeks or months of altered dietary habits (2, 3, 6, 8). The data do not support the theory (1) that the triglyceride rich chylomicrons appearing in plasma in the post alimentary phase take part in the formation of the fat emboli.

Dog B4, for some reason, did not absorb the coconut butter. It is therefore remarkable that this dog showed the most pronounced fat embolism in these series. If the fat emboli found in this dog's pulmonary circulation came entirely from the fat present in the plasma at the time of the injury, then it can be calculated that about 8 liters should have been needed.

The striking similarity in triglyceride composition between the fat emboli and bone marrow fat, on the contrary, supports the theory that the adipose tissue fat is the source of the emboli. The results do not even indicate that plasma lipoproteins to a small extent stick to the embolised fat in the pulmonary capillaries. This question will, however, be studied further by isotopic labelling of plasma triglycerides. The possibility that the emboli are synthetized from FFA released from the adipose tissue by the traumatic injury, as discussed earlier (5, 7), will also be the subject of further investigations.

ACKNOWLEDGEMENT

The study was supported by the Swedish Medical Research Council, Försvarsmedicinska sektionen (Projekt 40X 998).

The lipoprotein separations were performed by Anders Gustafson, M.D., to whom we are very grateful.

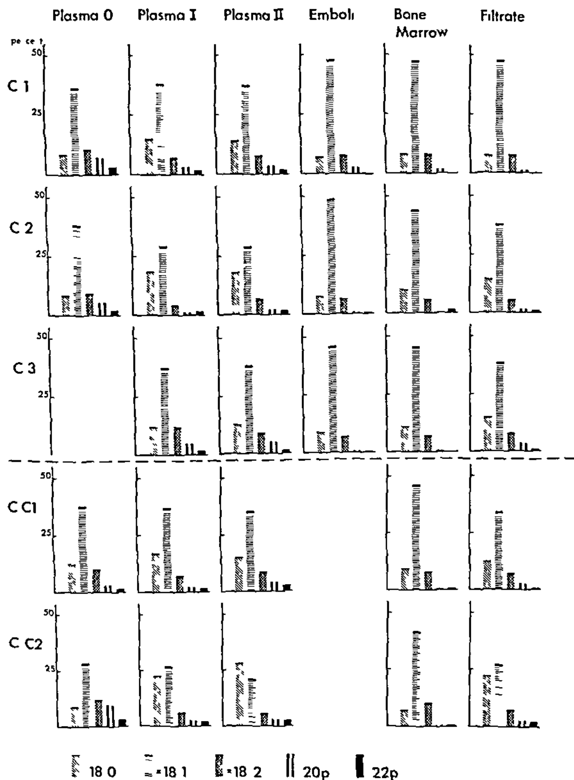


Fig 2 The percentage of 18 0 18 1 18 2 20 *p* and 22 *p* fatty acids of the triglycerides from plasma (Plasma 0) before the administration of tristearate before the *fracture* (*Plasma I*) and before the perfusion (Plasma II) compared to the composition in emboli bone marrow and filtrate Plasma 0 in dog *C3* and the emboli fractions in the non traumatized dogs (CC1 and CC2) were not analysed

PEFEPENCES

1 Bergentz, S E Stud es on the gene s of po t traumat c fat embol sm. Acta ch r scand. Suppl. s 1961

2 Brandon, J H & Karmen A The fatty ac d compos t on of chylomicrons of chyle and serum following the ingest on of different o ls J L p d. Pes 1 16 19 9 1960

3 Dayton, S Ha him to S D xon W & P arce M L. Compos t n of l p ds in human serum and adipose t sue during prolonged feeding of a d et h gh in unsaturated fat J Lip d. Pes 1(3 196(

4 Gustafson A. Stud es on human serum very low dens ty l poprote ns Acta med scand Suppl 446 1966

5 Hallgren, B Kerstell J Pul nstam, C M & Svanborg A. A m thod for the isolat on and chem al analys s of pulmonary fat embol Acta ch r scand 13 613 1966

6 Hirsch J Fatt ac d patterns in human a l pose t ssue In Handbook of Phys ology Sect 5 Chapter 1 Waverly Press Balt more USA 1965

Kerstell J Hallgren B Pu len tam, C M & S anborg A The chemical compos t on of the fat embol in the post absorpt e dog Acta m d scand Suppl 499 3 1969

8 Shap ro B Tr lycer le metabol m In Handbook of Ph s ology Sect on 5 Chapt r Wa erl Pres Balt more USA 1965

9 Swank P L & Du ger G S Fat embol m Surg Gynec Obstet 98 641 1954

1 Swank, P L Clinsman W & Slorp P The product on of fat embolism in rabb ts by feeding h gh fat meal Surg Gynec Obstet 110 9 1960

FROM THE DEPARTMENT OF PEDIATRICS II, VASA SJUKHUS, THE DEPARTMENT OF SURGERY I, AND THE DEPARTMENT OF MEDICINE II, SAHLGRENSKA SJUKHUSET, UNIVERSITY OF GÖTEBORG, GÖTEBORG, SWEDEN

IV. THE INCORPORATION OF LABELLED CHYLOMICRONS INTO THE FAT EMBOLI IN THE POST-ALIMENTARY DOG

BY

J. KERSTELL

Abstract. Pulmonary fat emboli were isolated and analysed in 3 dogs which had received 100 μCi of glycerol-^{14}C-tripalmitate together with alimentary fat in order to determine if plasma triglycerides stick to and/or are incorporated into emboli droplets after bone fractures. The administration of labelled fat caused the appearance of chylomicrons in which the labelled fatty acids were present. *No radioactivity was found in the fat emboli. The results therefore indicate that none of these chylomicrons were included in the fat emboli.*

In previous investigations (5, 6, 7) the triglyceride fatty acid pattern was found to be similar in post-traumatic pulmonary fat emboli and bone marrow fat but differed from the triglycerides in plasma of post-absorptive and post-alimentary dogs. These observations indicate that the plasma triglycerides are not the source of the fat emboli. However, there are previous studies in the literature indicating that variations in the blood lipids influence the embolisation (1, 10, 11). In the present study a labelling of plasma triglycerides was performed in order to investigate if some small amount of plasma triglycerides stick to and/or are incorporated into the emboli droplets.

MATERIAL AND METHODS

Four dogs weighing about 20 kg were used in the present study. All dogs had been fasting overnight and received 200 ml of cream containing 70 g of fat mixed with 100 μCi of glycerol-^{14}C-tripalmitate (Radiochemical Centre, Amersham, England).

The experimental procedure was the same as that described previously (7). In dogs R1–R3 femoral fractures were produced 3–4 hours after the administration of cream. Dog PC1 was not traumatized. The isolation of the emboli and the histological and chemical analyses were made as described previously (7) with the exception that all lobes except the control lobe were included in the total lung perfusion and no perfusion of a separate lobe was undertaken.

Three to four hours after the fracture the pulmonary circulation was isolated and perfused in the retrograde direction with isotonic citrate solution. The perfusate was then passed through a filter with a pore size of 28 μ, centrifuged at 1400 g for 20 min at 5° C and finally passed through a new filter with a pore size of 28 μ. The main part of the emboli in the perfusate was caught in the filters.

The efficiency of the perfusions was analysed by comparison of the number of emboli in a non-perfused lobe with that in the perfused lobes. The histological sections were stained with osmic acid and the emboli counted in 6–9 cm² of lung tissue from each lobe.

Arterial blood samples were taken before the administration of cream (Plasma 0), immediately before the fracture or corresponding time (Plasma I) and before the perfusion of the pulmonary circulation (Plasma II). Bone marrow specimens were taken from the femoral diaphyses and adipose tissue specimens from the subcutaneous inguinal fat at the end of the ex-

periments. As in the previous experiments (7) the lipids in the filters were called "emboli" and those in the perfusate passing both filters "filtrate".

In dog P2 the serum lipoproteins were separated ultracentrifugally (4). The fractions obtained were analysed as the plasma fractions. The main lipid classes were separated by thin layer chromatography on silicic acid as described previously (7). After the development of the chromato-plates the individual spots were made visible by iodine vapour and the triglyceride spots demarcated on the chromato-plates. The plates were stored at room temperature for 6–12 hours to allow the iodine to evaporate. The triglyceride areas were then scraped down into counting vials containing 10 ml of a mixture of PPO* POPOP* Cab-O-Sil* toluene in the proportions of 0·4:0·03:4:100 (w/w/w/v) respectively as recommended by Snyder & Stephens (9). The radioactivity was measured in a liquid scintillation spectrometer (Packard Tri

* Packard Instrument Company, USA.

Table I. The amount of triglycerides (TG) and their specific activity in the various fractions in three traumatized (P1–P3) and in one non traumatized dog (PC 1) which had received labelled triglycerides together with cream. Plasma samples were taken before the administration of cream (Plasma 0), before the trauma (Plasma I) and before the perfusion (Plasma II). Triglycerides in the perfusates from the lung vessels passing both filters were called "filtrate". "tr" stands for less than 10 cpm/mg of triglycerides.

	Amount of TG in the fraction*				Specific activity (cpm/mg triglyceride)			
Dog	P1	P2	P3	PC1	P1	P2	P3	PC1
Plasma 0	43	58	42	35	tr	tr	0	tr
Plasma I	71	160	57	44	1308	1634	138	1086
Plasma II	62	31	58	53	1434	606	237	1068
Emboli	205	15	65	1	tr	tr	0	100
Filtrate	45	21	60	20	258	301	33	1116
Bone marrow					tr	26	0	24
Subc. fat					tr	tr	–	tr

* In plasma mg/100 ml, in emboli and filtrate mg/fraction.

Table II. The amount and the specific activity of the triglycerides in the various serum lipoprotein classes in a sample taken immediately after plasma sample I and before the traumatic injury in dog P2. VLDL = Very low-density Lipoproteins, LDL = = Low-density Lipoproteins, HDL = High-density Lipoproteins.

	Lipoprotein class				
	Chylomicrons (S_f > 400)	VLDL (S_f 20–400)	LDL (S_f 0–20)	HDL	Plasma I
Triglycerides mg/100 ml	107	35	22	9	160
Specific activity cpm/mg of triglyceride	2076	963	154	190	1634

Carb model 3314). Correction for quenching was performed with the aid of external standard method. In some experiments this method was compared with an internal standard method in which ^{14}C toluene was used. This comparison showed a good correlation between the two methods. About 2 mg of triglycerides from each fraction were used in the determinations. Each sample was counted for 10 or 20 min. More than 2000 counts above the background were counted from the plasma samples taken after the administration of the labelled triglycerides.

RESULTS

The control lobe contained in dog R1 9, in dog P2 8 and in dog P3 16 emboli/cm of lung tissue.

The number of emboli left in the perfused lung lobes was 30–50 per cent of that of the control lobe. In the non traumatized animal only a few emboli were observed. The amount of lipids in the various fractions are shown in Table I.

Despite a high radioactivity in the plasma triglyceride fractions no significant radioactivity was detected in triglycerides in the emboli, bone marrow or subcutaneous fat tissue in the traumatized dogs (Table I).

In the dog in which the lipoprotein classes were separated by an ultracentrifugal technique the highest activity was found in the chylomicron fraction (Table II).

DISCUSSION

The observation that in a post alimentary state the intake of labelled digestible triglycerides results in a considerable labelling of plasma triglycerides due to the appearance of chylomicrons with a high content of the ingested triglyceride is in agreement with earlier findings (3). As could be expected (2, 8) the radioactivity of the bone marrow and subcutaneous fat tissue was found to be negligible 6 hours after the fat meal. As no significant radioactivity was found in the emboli triglycerides the results indicate that almost no plasma triglycerides were included in the emboli.

Triglycerides with different fatty acid compositions should vary in their physical properties. Therefore in these series of studies concerning the genesis of fat emboli the possible contribution of chylomicron triglycerides with different fatty acid composition including mostly C12–C14 fatty acids (6), mostly saturated C18 fatty acids (6) and in the present study saturated C16 fatty acids has been investigated.

As none of these chylomicrons were found to be included in the emboli the assumption that the emboli are formed from the chylomicrons, or that the chylomicrons stick to the emboli which come from other origin, is not supported by the results of these investigations.

ACKNOWLEDGEMENT

The study was supported by the Swedish Medical Research Council, Forsvarsmedicinska sektionen (Projekt 40X 998).

The lipoprotein separations were performed by Anders Gustafson, M.D., to whom I am very grateful.

REFERENCES

1. Bergentz, S. E.: Studies on the genesis of posttraumatic fat embolism. Acta chir. scand. Suppl. 282, 1961.
2. Dayton, S., Hashimoto, S., Dixon, W. & Pearce, M. L.: Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat. J. Lipid Res. 7:103, 1966.
3. Fredrickson, D. S., Levy, R. I. & Lees, R. S.: Fat transport in lipoproteins — an integrated approach to mechanisms and disorders. New Engl. J. Med. 276:34, 1967.
4. Gustafson, A.: Studies on human serum very low-density lipoproteins. Acta med. scand. Suppl. 446, 1966.

5 Hallgren, B Kerstell J Pudenstam C M & Svanborg A A method for the isolat on and chemical analysis of pulmonary fat emboli. Acta chir scand 134 613 1968
6 Hallgren, B., Kerstell J., Pudenstam, C M & Svanborg A The chemical composition of the fat emboli in the post alimentary dog Acta med. scand. Suppl 499.25 1969
- Kerstell, J., Hallgren, B Pudenstam, C M & Svanborg A The chemical composition of the fat emboli in the post absorptive dog Acta med. scand Suppl. 499.3 1969
8 Shapiro L Triglyceride metabolism In Handbook of Physiology Sect on 5 Chapter 22 Waverly Press, Baltimore USA, 1965
9 Snyder F & Stephens N Quantitative carbon 14 and tritium assay of thin layer chromatography plates Anal t Biochem. 4 128 1962
10 Swank, R L & Dugan G S Fat embolism. Surg Gynec Obstet 98 641 1954
11 Swank, R L. Glinsman W & Sloop P The production of fat embolism in rabbits by feeding high fat meal Surg Gynec Obstet 110-9 1960

FROM THE DEPARTMENT OF GERIATRICS II, VASA SJUKHUS, THE DEPARTMENT OF SURGERY I, AND THE DEPARTMENT OF MEDICINE II, SAHLGRENSKA SJUKHUSET, UNIVERSITY OF GÖTEBORG, GÖTEBORG, SWEDEN

V. THE CHEMICAL COMPOSITION OF THE FAT EMBOLI IN DOGS WITH EXTIRPATED LIVER AND INTESTINES

BY

J. KERSTELL

Abstract. The formation and composition of fat emboli were studied in traumatized dogs in which the synthesis of lipoproteins was eliminated by extirpation of the liver and intestines. Neither the amount of the emboli nor the composition of the emboli differed from those found in earlier experiments in dogs with these organs intact. The results indicate, therefore, that the fat emboli are not formed from FFA released by the trauma and then reesterified in the liver and intestines.

In the previous studies in these series it was shown that fat emboli have a composition similar to that of adipose tissue triglycerides, indicating that the source of the emboli fat in traumatized dogs (femoral fractures) is the adipose tissue (5, 6, 9). However, this does not necessarily mean that the emboli are mechanically liberated from the traumatized fat. The possibility that the increased mobilisation rate of FFA from adipose tissue caused by the traumatic injury (11) is involved in the emboli formation must be considered (10). The adipose tissue triglycerides seem to be released only as free fatty acids (FFA) under normal conditions (2). There is no convincing evidence indicating release in the form of tri-, di- or monoglycerides. The released FFA must then be reesterified before taking part in the emboli formation. Many tissues are able to incorporate FFA into triglycerides (3, 8), but only the triglycerides synthesized in the liver (4, 7, 8) and the intestine (1, 3) are released into the blood stream, where they appear in the lipoproteins. In order to elucidate the role of the liver and the intestines in the formation of fat emboli after traumatic bone injury, these organs were extirpated in dogs subjected to femoral fractures. Qualitative and quantitative analyses of the fat emboli were then performed.

MATERIAL AND METHODS

Five dogs weighing 17–23 kg were used. They had been fasting overnight. The dogs were anesthetized intravenously with pentobarbital sodium and the liver and intestines were then removed. Immediately thereafter bilateral femoral fractures were produced in dogs F1–F3 with a pair of pipe tongs. No fractures were produced in dogs FC1–FC2.

In all dogs symptoms of hypovolemia were observed after the abdominal operation. The dogs had tachycardia, irregular respiration and a drop in blood pressure was also noted. The hypovolemia was treated with 1000–2000 ml of isotonic saline administered during the experimental period.

Three to four hours after the fracture the pulmonary circulation was isolated and perfused in the retrograde direction with isotonic citrate solution. The perfusate was then passed through a filter with a pore size of 28 μ, centrifuged at 1400 g for 20 min at 5° C and finally passed through a new filter with a pore size of 28 μ. The main part of the emboli in the perfusate was caught in the filters.

The efficiency of the perfusions was analysed by comparison of the number of emboli in a

non perfused lobe with that in the perfused lobes. The histological sections were stained with osmic acid and the emboli counted in 6–9 cm² of lung tissue from each lobe.

The methods for the isolation of the fat emboli and for the chemical and histological analyses have been described previously in detail (9).

Blood sampling was performed before the start of the abdominal operation (Plasma I) and *immediately before the perfusion procedure* (Plasma II). As in earlier experiments (9) the filter fractions were denoted "emboli" and the perfusates passing both filters "filtrate".

The statistical calculations were carried out as given in previous reports (9).

RESULTS

In dog F1 the number of emboli was 2, in F2 35 and in F3 1/cm² of lung tissue. In the control lobe the mean number remaining in the lung lobes was on an average 40, 10 and 10 per cent respectively of that of the non perfused lobe. In the animals without fractures (F C1–F C2) only a few emboli were observed but the general impression was that the number of emboli was somewhat higher than in the animals without fractures in the previous studies in these series (6, 9).

The amount of triglycerides, cholesterol and phospholipids in plasma, emboli and filtrate fractions is shown in Table I. The levels of the plasma lipids decreased markedly in all animals during the experiments. The amount of fat in the emboli fraction in the traumatized dogs, was within the same range as that in animals with intact liver and intestines studied earlier (6, 9) but slightly higher in the animals without fractures in the present experiments. *The fatty acid composition of the* triglycerides in different fractions is given in Table II and illustrated for the main fatty acids in fig. 1.

The plasma triglycerides changed somewhat during the experiments. Most of the changes in these percentage compositions were small. The percentage of C22:0 increased in all animals. This increase in C22:0 was found to be inversely related to the triglyceride level in plasma in the sample before the perfusion (Plasma II). A comparison between the triglycerides in plasma, bone marrow fat and fat emboli revealed a striking similarity between bone marrow and emboli triglycerides. In Table III the correlations between the fatty acids of the triglycerides in emboli and plasma and between emboli and bone marrow triglycerides are given. A closer similarity between emboli and bone marrow than between the emboli and plasma triglycerides was observed.

Table I. Triglycerides (TG), cholesterol (Chol) and phospholipids (Plip) in the plasma (mg/100 ml) before the fracture (Plasma I) and before the perfusion (Plasma II). The lipid values for the emboli and *filtrate are given as mg in the fractions*

Dog	Plasma I			Plasma II			Emboli			Filtrate			Filtrate amount plasma TG calculated from	
	TG	Chol	Plip	TG	Chol	Plip	TG	Chol	Plip	TG	Chol	Plip	Hb	Plip
F1	14	150	285	1	15	35	14	0.7	1.9	2	16	25	1	1
F2	24	130	232	7	33	58	183	0.8	3.4	29	23	32	3	4
F3	20	206	346	5	73	118	36	1	1.2	6	31	43	1	2
F C1	26	103	194	17	74	125	1.2	0.5	0.6	5	21	37	5	5
F C2	20	132	[illegible]	3	22	43	1.4	0.4	0.7	1	9	13	1	1

Table II The fatty acid composition of the triglycerides *in plasma before* the fracture (PI) and before the perfusion (PII) fat emboli *fraction* (E) bone marrow (BM) and *filtrate* (F) in the traumatized dogs (F1–F3) and the dogs without fractures (FC1–FC2)

Fatty acid	F1					F2					F3					FC1				FC2			
	PI	PII	E	BM	F	PI	PII	E	BM	F	PI	PII	E	BM	F	PI	PII	BM	F	PI	PII	BM	F
12	1.1	0.6	1.3	1.0	1.7	0.3	1.0	1.4	0.8	0.8	0.3	1.2	1.1	0.8	1.9	0.[illegible]	0.7	0.8	0	0.8	0.9	0.3	1.5
14	1.7	3.1	4.2	3.9	3.5	1.6	2.3	4.3	4.1	3.4	2.0	3.1	4.6	3.7	4.5	2.9	2.4	[illegible].8	1.9	2.2	3.3	3.1	3.3
15	0.6	1.2	0.5	0.5	0.5	0.6	1.2	0.9	0.4	0.7	0.3	1.2	0.6	0.7	1.3	1.1	0.7	0.5	0.2	1.2	1.1	0.2	1.2
16:0	26.0	22.5	23.0	23.7	21.0	24.4	19.9	22.5	23.1	21.6	22.2	19.8	23.8	22.7	22.1	23.3	22.5	29.3	22.3	21.8	21.8	22.7	20.5
16:1	1.9	2.6	3.1	2.6	2.8	1.5	2.3	4.5	3.7	3.6	2.9	3.2	3.5	3.5	3.9	2.2	1.8	3.6	1.7	2.4	2.6	4.1	3.5
17	1.2	1.7	0.8	0.8	1.2	2.7	2.8	1.1	0.9	1.0	1.1	2.4	0.9	1.3	2.1	1.6	1.4	0.7	1.7	3.4	3.8	0.7	2.1
18:0	9.6	8.1	6.9	8.4	6.8	7.6	5.7	5.8	6.3	7.2	9.1	6.1	7.8	7.8	8.4	9.4	9.4	5.8	9.0	8.0	7.6	6.9	7.0
18:1	29.9	22.9	43.7	41.8	39.5	41.2	4[illegible].0	46.5	48.0	45.3	33.4	40.0	42.3	42.9	37.0	3[illegible].9	38.[illegible]	42.0	38.6	3[illegible].4	30.2	49.6	35.6
18:2	13.1	5.7	8.8	8.3	8.8	8.9	6.[illegible]	7.4	7.1	8.0	12.9	11.0	9.4	9.9	9.6	10.2	9.2	7.[illegible]	10.4	10.3	8.2	9.9	9.[illegible]
19	0.2	0	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.4	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.3	0.4	0.2	0
20:0	0.3	0.9	0.6	0.7	0.5	0.2	0.3	0.4	0.3	0.4	0.3	0.7	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.2	0.4
20:1	1.2	0.9	3.8	4.1	2.7	1.2	1.1	2.2	2.0	2.4	1.4	1.0	2.4	2.7	1.9	1.3	1.4	1.0	1.2	0.7	0.[illegible]	1.0	1.3
20p	4.9	1.3	0.6	0.5	1.8	5.1	5.7	0.5	0.3	0.7	7.8	2.7	0.8	0.6	2.8	4.9	5.[illegible]	0.6	4.5	4.0	4.6	0.5	3.1
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22:0	7.2	27.1	1.3	1.7	7.2	2.7	3.5	1.8	1.6	3.5	3.0	6.6	1.3	1.4	2.5	[illegible].3	2.6	1.3	5.4	7.8	12.9	1.3	3.8
22:1	0.6	1.1	1.5	1.5	1.4	0.5	0.2	0.9	1.0	1.0	0.7	0	0.9	1.0	0.7	0.5	0.5	0.4	0.5	0.2	0.7	0.2	0.5
22p	1.5	0.3	0.2	0.2	0.4	1.2	1.8	0.1	0.2	0.2	2.4	0.6	0.2	0.3	0.6	3.0	2.9	0.2	2.1	1.2	1.0	0.1	1.0

Table III The correlations in the fatty acid percentages in the triglycerides emboli vs plasma and emboli vs bone marrow The correlations were compared by Snedecor's F test applied to the residual variances (s^2_{yx}) * indicates that the relationship between emboli vs bone marrow is closer ($p < 0.01$) than emboli vs both plasma I and II

	Emboli ~ Plasma I		Emboli ~ Plasma II		Emboli ~ Bone marrow	
Dog	r	s_{yx}	r	s_{yx}	r	s_{yx}
F1	0 93	4 1	0 68	8 5	1 00	0 64*
F2	0 98	2 2	0 99	1 8	1 00	0 30*
F3	0 97	2 9	0 99	1 9	1 00	0 44*

DISCUSSION

The extirpation of the liver and intestines prevented neither emboli production nor did it cause significant embolisation in the animals without fractures On the contrary the amount of triglycerides in the emboli fraction washed out was in the same order of magnitude as in previous experiments in dogs with these organs intact In dog F2 the total amount of triglycerides disappearing from plasma during the experimental period was calculated to be about 130 mg The blood loss caused by the operation was estimated to be at least 30 per cent of the initial blood volume The amount of plasma triglycerides available for fat embolisation would thus have been about 100 mg In the lung vessels however, the calculations revealed no less than 240 mg of emboli triglycerides The plasma triglycerides therefore could not possibly be the sole source of the emboli in this dog

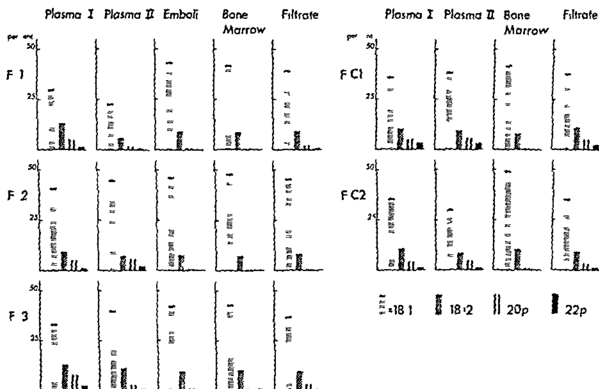


Fig 1 The percentages of 18 1 18 2 20 p and 22 p fatty acids in triglycerides in plasma before the extirpation of liver and intestines (Plasma I) before the perfusion (Plasma II) emboli bone marrow and filtrate FC1 FC2 are dogs in which no fractures were produced

Furthermore, the fatty acid composition in the present experiments in which the possibility of triglyceride and lipoprotein synthesis in liver and intestines had been excluded, was also similar in bone marrow and emboli but differed between plasma and emboli triglycerides

The present results therefore, further strongly support the theory that fat emboli after bone injury originate from mechanically liberated fat droplets in the traumatized area

ACKNOWLEDGEMENT

The study was supported by the Swedish Medical Research Council Forsvarsmedicinska sektionen (Projekt 40X 998)

The gas liquid chromatography analyses were performed in Astra Nutrition laboratories

REFERENCES

1 Bierman E I Particulate lipid components in plasma In Handbook of Physiology Section 5 Chapter 51 Waverly Press Baltimore USA 1965

2 Carlson L A Boberg J & Hogstedt B Some physiological and clinical implications of lipid mobilization from adipose tissue In Handbook of Physiology Section 5 Chapter 63 Waverly Press Baltimore USA 1965

3 Carlson L A Liljedahl S O & Wirsen C Blood and tissue changes in the dog during and after excessive free fatty acid mobilization Acta med scand 178 81 1965

4 Fredrickson D S Levy R I & Lees P S Fat transport in lipoproteins — an integrated approach to mechanisms and disorders New Engl J Med 276 34 1967

5 Hallgren B Kerstell J Rudenstam C M & Svanborg A A method for the isolation and chemical analysis of pulmonary fat emboli Acta chir scand 132 613 1966

6 Hallgren B Kerstell J Rudenstam C M & Svanborg A The chemical composition of the fat emboli in the post alimentary dog Acta med scand suppl 499 25 1969

7 Havel J P Conversion of plasma free fatty acids into triglycerides of plasma lipoprotein fractions in man Metabolism 10 1031 1961

8 Havel J R & Goldfein A The role of the liver and extrahepatic tissues in the transport and metabolism of fatty acids and triglycerides in the dog J Lipid Res 2 389 1961

9 Kerstell J Hallgren B Rudenstam C M & Svanborg A The chemical composition of the fat emboli in the post absorptive dog Acta med scand Suppl 499 3 1969

10 Liljedahl S O & Westermark L Aetiology and treatment of fat embolism Acta anaesth scand 11 177 1967

11 Wadstrom L B Effects of trauma on plasma lipids an experimental study in the rat Acta chir scand 115 409 1958

These

of bone

The co
m nary
found to
marrow
from ot
source
present
as to
liberate
drople
rate o
by t

FROM THE DEPARTMENT OF GERIATRICS II, VASA SJUKHUS, THE DEPARTMENT OF SURGERY I, THE DEPARTMENT OF MEDICINE II AND THE CENTRAL LABORATORY, SAHLGRENSKA SJUKHUSET, UNIVERSITY OF GOTEBORG, GOTEBORG, SWEDEN

VI. THE INFLUENCE OF INCREASED AND DECREASED PLASMA FREE FATTY ACIDS ON THE FORMATION AND COMPOSITION OF THE FAT EMBOLI IN THE DOG

BY

B. HALLGREN, J. KERSTELL, C. M. RUDENSTAM and A. SVANBORG

Abstract. Previous observations in these series of studies in dogs with femoral fractures showed that pulmonary fat emboli have a triglyceride fatty acid composition similar to bone marrow fat.

The present study is concerned with the question as to whether the emboli are mechanically liberated from traumatized fat rich tissues or the result of increased mobilisation rate of free fatty acids incorporated into triglycerides outside the adipose tissue.

Variations in the fatty acid level caused by the administration of norepinephrine or nicotinic acid did not influence the number of pulmonary fat emboli, the amount of emboli triglycerides or their fatty acid composition. No significant correlations were observed between the plasma FFA level and the number or amount of pulmonary fat emboli.

^{14}C labelled fatty acids bound to albumin were infused during the experiments in 3 dogs simultaneously infused with norepinephrine. These fatty acids were not incorporated in the fat emboli.

These observations indicate that fat embolisation after fractures in dogs is due to a mechanical liberation of bone marrow fat and not to the rise in the rate of fatty acid mobilisation from the adipose tissue induced by the trauma.

The composition of the triglycerides in pulmonary fat emboli after fractures has been found to be very similar to that of the bone marrow fat, indicating that this fat or fat from other parts of the adipose tissue is the source of the emboli (6, 7, 8, 10, 11). The present report is concerned with the question as to whether the emboli are mechanically liberated from traumatized fat rich tissues as fat droplets or the result of increased mobilisation rate of free fatty acid (FFA) (1, 2, 12) induced by the traumatic injury, and secondary synthesis of fat emboli triglycerides outside the adipose tissue. As fat emboli occur after fractures in dogs with extirpated liver and intestines (10) this secondary synthesis must occur in organs other than liver or intestine. Using the previously described technique for the isolation and analyses of pulmonary fat emboli after fracture in dog (11), the influence of extreme variations in the rate of FFA release from adipose tissue on the amount and composition of fat emboli was studied.

MATERIAL AND METHODS

Fifteen dogs weighing 12–25 kg were used in these experiments. Food had been withheld overnight. They were anesthetized intravenously with pentobarbital sodium.

In dogs D1–D6 and E1–E5 bilateral femoral fractures were produced with a pair of pipe tongs. Dogs D1–D6 received 2–3 μg/min./kg body weight of norepinephrine (Nor Exadrine®) during the whole experiment. The norepinephrine was added to 1000 ml 0.9 per cent saline and administered as a drip infusion. In dog D2 the infusion was started 2 hours before and in the other dogs immediately after the trauma. A drop in blood pressure was observed in dogs D1–D4, which necessitated the administration of additional saline (500–1500 ml).

Dogs E1–E5 received 5 mg/kg of nicotinic acid (Nikotinsyra ACO) intravenously every 10 min. for 2–3 hours before the fracture and 3–4 hours thereafter until the lung vessels

were perfused Dogs D C1–C3 served as non traumatized controls for the norepinephrine treated group They were otherwise handled as the traumatized animals Due to technical reasons the rate of the norepinephrine infusion could not be kept constant in dog DC3 In the non traumatized dog E C1 5 mg/kg body weight of nicotinic acid was administered every 10 minutes for 6 hours

The isolation of the emboli and the chemical analyses including the triglyceride fatty acid determination have been described previously in detail (11) In some experiments the composition of the plasma FFA fraction was also determined In two dogs (D1 and D C2) the lower right lobe was at first perfused separately In the other animals all four main lobes except the control lobe were included in the total lung perfusion Three to 5 hours after the fracture the pulmonary circulation was isolated and perfused in the retrograde direction with isotonic citrate solution The perfusate was then passed through a filter with a pore size of 28μ centrifuged at 1400 g for 20 min at 5 C and finally passed through a new filter with a pore size of 28μ The main part of the emboli in the perfusate was caught in the filters The efficiency of the perfusions was analysed by comparison of the number of emboli in a non perfused lobe with that in the perfused lobes The histological sections were stained with osmic acid and in some dogs also with *Scarlet Red and the emboli counted in 6–9 cm²* of lung tissue from each lobe

In dogs D1–D6 and DC1–DC3 30–40 ml of blood was taken before the fracture or corresponding time (Plasma I) and before the perfusion (Plasma II)

In dogs E1–E5 and EC1 30–40 ml of blood was taken before the administration of nicotinic acid (Plasma 0) before the fracture or in the non traumatized animal after corresponding time (Plasma I) and before the perfusion (Plasma II) Ten ml of blood was drawn every 30 min for the FFA analyses during the whole experiments in all dogs The last sample was drawn when the norepinephrine infusion was stopped or a few minutes later in dogs D1–D6 and dogs DC1–DC3 Bone marrow samples were taken from the femoral diaphyses FFA was determined according to Duncombe (4)

The solvent in an ampoule containing 0 1 mCi of ^{14}C oleic acid, Radiochemical Centre Amersham, England, was evaporated under nitrogen Three ml of absolute ethanol containing 0 5 ml of 0 02 N KOH was added and dried just to dryness on a water bath The contents of the bottle were then dissolved in 10 ml of 20 per cent human serum albumin (Kabi Sweden) and then diluted to about 80 ml with 0 9 per cent saline In dog D4–D6 0 025–0 05 μCi/min /kg body weight of the ^{14}C oleic acid bound to human albumin were infused with constant rate during the experiment with the start of the infusion 20–35 min before the fracture Five—10 ml of blood was also drawn through a catheter in the right ventricle (dog D4) or inferior caval vein (dogs D5–D6) every 30 min The main lipid classes were separated during thin layer chromatography and the radioactivity in the plasma FFA and triglycerides was estimated as described previously (8)

As in previous experiments (11) the filter fractions were denoted emboli and the perfusate passing both filters 'filtrate The statistical calculations were preformed as earlier (11)

RESULTS

In the group treated with norepinephrine dogs D1–D4 showed very large fracture hematomas and more pronounced bleeding than the other dogs in this and in our previous investigations (6 7 8 10 11) They also had irregular respiration tachycardia and cardiac arythmias In dog D5 only transverse fractures with very smooth margins of the fragments were obtained and almost no fracture hematomas were observed Both dog D5 and D6 tolerated the drug and the trauma well In two of the dogs treated with norepinephrine blood pressure was determined There was an initial rise of about 50 mm Hg whereafter the pressure fell to about or just below the initial values and then remained at

this level The dogs receiving nicotinic acid tolerated this treatment well

All traumatized dogs showed pulmonary fat emboli with the exception of dog D5 in which almost no emboli were observed (Table I) In the control animals fat emboli were seen only occasionally The mean number of emboli remaining in the perfused lobes is also given in Table I The number of emboli remaining in the perfused lobes was lower in the non perfused control lobes in all animals except in dog D5 and E5

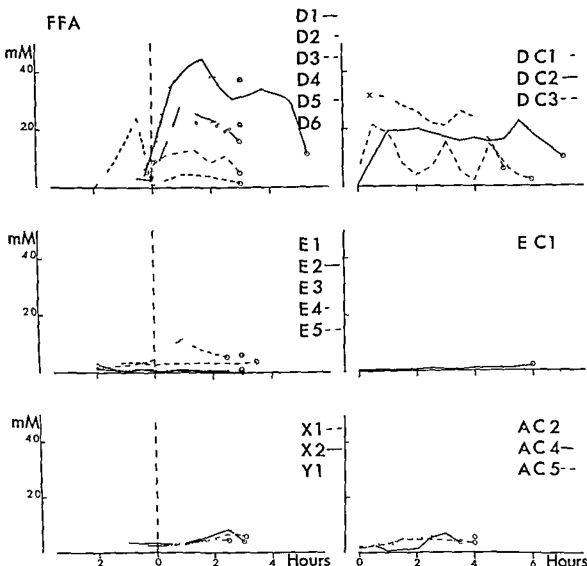
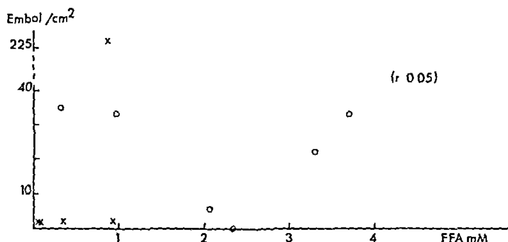


Fig 1 The arterial FFA level during the treatment with norepinephrine or nicotinic acid The perpendicular dotted line indicates the fracture o indicates the sample immediately before the perfusion In dogs D1 D3–D6 the norepinephrine infusion was started after the trauma in dogs D2 and DC1–DC3 after the first FFA sample The first FFA sample was lost in dog DC1 but a new one was taken a few minutes after the trauma and after the start of the norepinephrine infusion (×) Due to technical reasons the rate of the norepinephrine infusion could not be kept constant in dog DC3 In dogs E1–E5 and EC1 the nicotinic acid administration was started after the first FFA sample Dogs X1 X2 and Y1 (traumatized animals) and AC2 AC4 AC5 (non traumatized dogs) were not treated with any of the drugs

Table I The number of embol /cm² n the control and perfused lobes (mean values) n the traumat zed dogs Dog D1 D6 had rece ved norep nephr ne infus on and dog E1 E5 n cot n c a d dur ng the exper ment

Dog	Control lobe embol /cm²	Perfused lobes embol /cm²
D1	º	1
D	35	11
D3	34	º4
D4	34	13
D5	a fe	a f ε
D6	6	0 6
E1	º	0 3
E	º	1 º
E3	º	1 3
E4	º 7	34
E5	º	3

The norepinephrine infusion caused an elevation of the plasma FFA level in both traumatized animals and controls (Fig 1) However in dog D2 in which the infusion started 2 hours before the trauma the FFA level ro e only initially and returned to about the pre experimental level after the fracture As far as we observed there was no technical failures with the infusion The dog also reacted very markedly to the infusion In dogs E1 L2 and E5 no post traumatic increase in the FFA level occurred after the administration of nicotinic acid but an increase was observed in dogs E3 and E4 (fig 1) Figure 1 also includes FFA analyses in 3 traumatized (A1 A2 A1) and 3 non traumatized dogs (A C2 A C4 A C5) who had not received norepinephrine or nicotinic acid These experiments



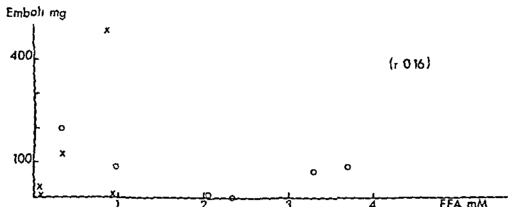


Fig º The correlat ons bet een the number of embol and the amount of embol fat washed out s th m an FFA level dur ng the per od after the trauma n 11 dogs (D1 D6 o and E1 E5 (x))

are described in other publications (5 9, 10) The changes in FFA induced by the trauma were much less pronounced than the changes caused by norepinephrine

When the number of emboli and the amount of emboli fat were plotted against the mean FFA level during the period after the fracture no correlation was found (fig 2)

The amount of lipids in the plasma, emboli and filtrate fractions is shown in Tables II and III The triglyceride fatty acid composition in plasma was not generally influenced by the administration of the drugs during the period studied (Table IV) The fatty acid composition of the emboli triglycerides was similar to that of bone marrow and differed from that of plasma triglycerides (Table IV, fig 3 and 4) The correlation between the fatty acid pattern in emboli vs plasma and emboli vs bone marrow is shown in Tables V and VI

The fatty acid pattern in the arterial FFA fraction differed from the pattern obtained from bone marrow triglycerides (*Dogs D3* and D C1–D C3) The FFA fraction contained more C16 0 and C18 0, and less C18 1 fatty acids (Table VII) Table VII shows also the com positions of the arterial plasma FFA in 3 dogs in earlier experiments (7 11) one postabsorptive dog, A4, (11) one dog B1 which had received coconut butter (7) and one dog C1, which had received a synthetic tristearate preparation (7) Similar differences between plasma FFA and bone marrow triglycerides were observed in these animals The fatty acid pattern in the emboli triglycerides in the traumatized animals (A4 B1, C1 and D3) is also presented

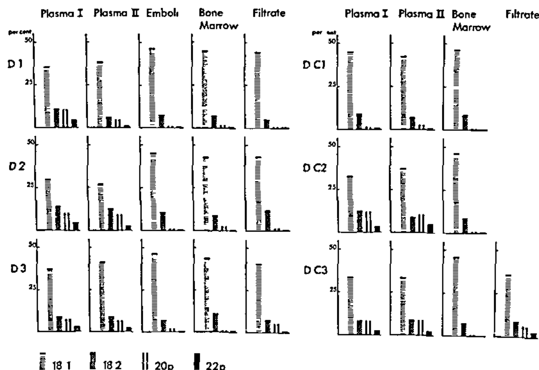


Fig 3 The percentages of 18 1 18 2 20 *p* and 22 *p* fatty acids in the triglycerides in plasma before the fracture or corresponding time (Plasma I) and before the perfusion (Plasma II) emboli bone marrow and filtrate in 3 traumatized (D1–D3) and 3 non traumatized dogs (DC1–DC3) treated with norepinephrine

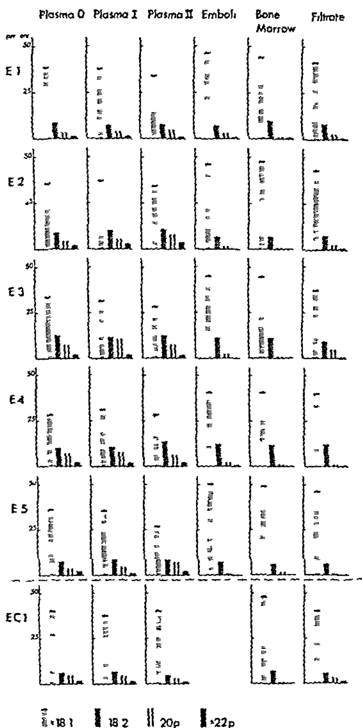


Fig 4 The percentages of 18 1 18 2 20 *p* and 22 *p* fatty acids of the triglycerides in pla ma emboli bone marrow and filtrate in 5 traumatized (E1 E5) and one non traumatized dog (EC1) Plasma samples were taken before the administration of nicotinic acid (Plasma 0) before the fracture or corresponding time (Plasma I) and before the perfusion (Plasma II)

Table II Triglycerides (TG) cholesterol (Chol) and phospholipids (Plip) in the plasma (mg/100 ml) before the fracture and before the perfusion in the dogs treated with norepinephrine The amount of lipids (mg) in emboli and filtrate fractions Dogs DC1–DC3 were non traumatized dogs

	Plasma I			Plasma II			Emboli			Filtrate			Filtrate amount plasma TC calculated from	
Dog	TG	Chol	Plip	TG	Chol	Plip	TG	Chol	Plip	TG	Chol	Plip	Hb	Plip
D 1	11	252	444	14	228	410	76	2	2	31	96	150	5	5
D 2	27	256	212	19	152	220	231	3	1	117	41	61	6	5
D 3	28	210	349	15	102	175	91	0 5	2	22	52	92	6	8
D 4	31	196	299	33	180	248	92	2	1	41	115	197	11	26
D 5	32	193	340	18	117	230	0 8	0 3	0 7	6	9	12	1	1
D 6	32	177	308	15	134	259	7	0 5	0 7	9	36	69	3	4
D C1	45	137	254	34	126	246	0 7	0 4	0 1	2	9	15	1	2
D C2	23	158	335	9	159	272	2	1	2	1	35	54	1	2
D C3	45	344	517	39	275	371	0 9	0 9	1	15	107	204	9	21

Table III Triglycerides (TG) cholesterol (Chol) and phospholipids (Plip) in the plasma (mg/100 ml) before administration of nicotinic acid (Plasma 0) before the fracture (Plasma I) and before the perfusion (Plasma II) The amount of lipids (mg) in the emboli and filtrate fractions Dog EC1 was not traumatized

	Plasma 0			Plasma I			Plasma II			Emboli			Filtrate			Filtrate amount plasma TG calculated from	
Dog	TG	Chol	Plip	TG	Chol	Plip	TG	Chol	Plip	TG	Chol	Plip	TG	Chol	Plip	Hb	Plip
F 1	35	143	217	19	125	198	22	119	227	4	0 5	0 7	6	53	98	6	10
F 2	38	171	311	34	157	293	24	165	272	28	0 9	0 8	5	37	30	1	3
E 3	25	159	337	17	148	311	17	129	247	8	0 6	0 7	11	63	122	5	8
E 4	30	126	244	17	114	211	17	99	183	484	0 6	1 5	154	42	77	4	7
E 5	40	94	214	34	111	236	13	99	177	126	0 5	1 1	10	26	40	1	3
E C1	43	251	460	34	231	441	49	224	385	0 2	0 8	0 7	5	26	51	3	6

The results from the dogs infused with labelled fatty acids bound to albumine (D4–D6) are given in Table VIII The figures show that there might have been some uptake of FFA in the lung tissue triglycerides but that there was almost no labelling of the emboli and bone marrow triglycerides

DISCUSSION

After the administration of norepinephrine the cause of the increase in the FFA level is known to be mainly the result of an increased rate of FFA release from the adipose tissue (2 3) We do not know the reason why the FFA level rose only initially but later on returned to the preexperimental level in dog D2 The observation that the administration of nicotinic acid in dogs E1 E2 and E5 abolished the expected influence of the traumatic injury on the FFA level (fig 1) is in accordance with the observation by Carlson and co workers (1) In dogs E3 and E4 nicotinic acid did not markedly counteract the rise of the FFA level indicating that this dose of nicotinic acid was too small to abolish the effects of sympathetic stimulation by the traumatic injury

Even when the FFA release was blocked

by nicotinic acid, fat embolisation occurred The results did not show any correlation between the FFA level and the number of emboli or the amount of triglycerides in the emboli fraction Neither did the variations in the FFA level influence the composition of the emboli triglycerides Furthermore, in the experiments in which the fatty acid composition of the arterial plasma FFA fraction was compared to the composition of the emboli triglycerides and with the bone marrow triglycerides, a similarity was observed between the bone marrow fat and the emboli but a difference existed between FFA and emboli triglycerides

The finding that no labelled fatty acids from the plasma FFA fraction were recovered in the emboli further strongly supports that the fat emboli are not produced from FFA mobilized from adipose tissue by the trauma

It was remarkable that the four dogs (D1–D4) treated with norepinephrine which showed pronounced fracture hematomas also showed unusually high numbers of fat emboli But it must also be pointed out that the highest number of emboli in this investigation was found in a dog (E4) treated with nicotinic acid

Thus the observations made in this as well as earlier reported results in these series (5, 6, 7, 8, 10 11) indicate that fat emboli after fractures in dogs are due to a mechanical liberation of bone marrow fat

ACKNOWLEDGEMENT

The study was supported by the Swedish Medical Research Council Forsvarsmedicinska sektionen (Projekt 40X 998)

Table IV The fatty acid composition of the triglycerides in plasma before the administration of nicotinic acid (dogs denoted E) (I 0) before the fractures (P I) and before the perfusion (P II) fat emboli fraction (E) bone marrow (BM) filtrates (F) in the traumatized dogs (D1–D3 E1 E5) and the non traumatized dogs (DC1–DC3 and EC1)

	D1					D2					D3				
	P I	P II	E	BM	F	P I	P II	E	BM	F	P I	P II	E	BM	F
12	0	0	0 6	1 1	0 2	0 3	0	0 2	0 3	0 2	0 6	0 3	0 6	0 9	0 3
14	2 1	2 6	3 3	3 9	2 7	1 7	2 4	3 0	3 2	2 6	2 6	2 3	3 9	4 0	3 1
15	0 3	0 3	0 3	0 5	0 2	0 8	0 8	0 2	0 2	0 1	0 6	0 7	0 4	0 4	1 0
16 0	21 4	24 8	23 7	24 4	26 9	22 6	26 9	24 8	25 6	24 7	22 8	23 5	25 1	25 8	26 0
16 1	3 4	2 7	3 8	2 7	2 9	1 7	1 5	2 9	2 9	2 7	2 4	2 5	2 8	3 1	2 5
17	1 1	1 0	0 7	0 8	0 6	1 7	2 0	0 4	0 4	0 3	2 9	1 8	0 9	0 7	1 1
18 0	5 4	11 1	6 6	7 1	8 0	7 4	9 2	7 6	7 7	8 0	5 0	3 9	6 2	5 5	6 6
18 1	36 0	38 3	46 7	44 9	44 7	30 1	27 2	45 7	42 8	43 5	37 2	41 2	45 8	44 2	40 0
18 2	11 6	6 1	7 7	7 1	5 1	14 0	12 3	10 3	9 1	12 2	9 8	8 8	7 0	10 6	7 2
19	0 4	0 6	0 3	0 4	0 5	0 3	0 6	0 2	0 3	0 1	0 5	0 3	0 4	0 2	0 5
20 0	0 2	1 9	0 5	0 5	1 6	0 3	1 6	0 3	0 3	0 3	0 3	0 2	0 3	0 2	0 4
20 1	0 9	2 2	2 1	2 5	2 4	1 1	1 6	1 5	1 5	1 4	1 5	1 3	1 9	1 2	1 7
20p	10 3	4 5	0 9	1 4	0 4	10 1	9 0	0 9	2 4	1 3	7 3	6 6	2 2	0 4	5 3
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22 0	2 6	2 0	1 5	1 3	2 6	2 6	2 5	1 2	1 7	1 5	2 5	2 5	1 0	0 8	1 4
22 1	0	0 8	1 0	1 1	0 6	0 8	0	0 7	0 9	0 6	0 4	0 3	0 6	0 3	0 4
22p	4 7	1 1	0 7	0 3	0 7	4 5	2 5	0 5	0 7	0 5	3 2	2 7	0 2	0 3	0 7

Table IV (cont.)

	D C1			D C2			D C3			
	P I	P II	BM	P I	P II	BM	P I	P II	BM	F
12	0 1	0 4	0 5	0 3	0 1	0 2	0 7	0 8	0 3	0 3
14	1 1	1 9	2 8	1 2	1 1	2 1	3 3	2 8	3 9	2 0
15	0 4	1 0	0 3	0 5	0 3	0	1 6	1 7	0 2	1 0
16 0	19 8	20 7	21 5	20 6	20 3	24 1	25 5	24 3	26 8	24 6
16 1	1 8	2 6	3 1	2 0	1 6	1 7	1 9	2 0	2 9	1 7
17	2 6	3 3	0 5	1 2	1 2	0 5	3 4	3 1	0 4	3 0
18 0	8 1	7 0	6 1	7 1	9 4	9 0	8 2	9 5	9 3	9 4
18 1	45 1	42 6	48 3	33 1	37 4	46 4	34 1	33 5	45 8	35 8
18 2	8 9	7 1	8 6	12 3	8 8	6 4	7 8	9 4	7 4	8 3
19	0 4	0 5	0 3	0 3	0 3	0 2	0 4	0 4	0 2	0 8
20 0	0 9	0 7	0 7	0 5	1 0	0 8	0 3	0 4	0 3	1 2
20 1	1 8	1 5	3 3	1 4	2 0	2 7	1 1	1 0	1 1	0 9
20*p*	1 6	2 3	0 6	11 8	10 3	0 7	9 0	8 5	0 6	5 9
21	0	0	0	0	0	0	0	0	0	0
22 0	4 0	4 8	2 3	3 0	2 0	1 3	0 7	0 9	1 1	2 6
22 1	2 3	2 4	3 0	1 2	0 3	0 7	0 4	0 4	0 4	0 2
22*p*	1 2	1 2	0 1	3 5	4 9	0 3	2 6	2 3	0 2	2 3

	E1						E2 (printed F2)					
	P 0	P I	P II	E	BM	F	P 0	P I	P II	E	BM	F
12	0 1	0 5	0 3	0 6	0 6	0 5	1 2	1 2	1 0	0 6	0 5	0 8
14	0 8	1 4	1 1	3 8	2 5	1 9	3 1	2 8	2 1	3 3	3 6	3 1
15	1 7	3 2	3 9	0 6	0 3	2 1	1 9	1 8	2 3	0 5	0 6	1 1
16 0	19 7	19 7	18 4	22 0	22 0	18 3	22 4	21 1	18 0	24 5	24 9	22 2
16 1	1 2	1 9	1 5	4 2	2 3	3 0	2 2	2 4	2 0	3 0	3 8	3 5
17	3 5	5 6	6 6	0 8	1 2	3 6	2 2	1 7	2 4	1 3	0 7	1 7
18 0	12 6	12 3	11 9	5 5	6 9	8 3	6 9	6 6	7 7	7 3	7 2	7 1
18 1	39 2	39 6	34 7	46 9	44 4	42 2	36 3	37 3	34 6	46 4	47 8	43 6
18 2	7 9	7 2	7 7	7 7	9 4	8 0	8 6	10 0	10 9	6 6	6 3	7 7
19	0 3	0 4	0 2	0 3	0 3	0 2	0 4	0 3	0 3	0 3	0 2	0 3
20 0	0 5	0 2	0 7	0 5	0 4	0 5	0 4	0 4	0 3	0 4	0 3	0 3
20 1	0 8	0 7	0 7	1 8	1 0	1 0	2 5	2 3	2 1	1 5	1 4	1 5
20*p*	2 8	4 1	4 8	2 9	1 1	2 3	4 3	5 3	7 8	1 4	0 4	2 3
21	0	0	0	0	0	0	0	0	0	0	0	0
22 0	[illegible]	2 8	3 6	1 5	5 0	5 6	4 2	2 9	4 6	2 6	1 6	3 4
22 1	[illegible]	0 3	2 8	0 6	1 5	2 0	1 2	1 0	1 0	0 5	0 5	0 5
22*p*	1 1	1 2	1 2	0 3	0 3	0 6	2 2	2 9	3 5	0 2	0 2	0 0

Table IV (cont)

	F3						E4					
	P 0	P I	P II	E	BM	F	P 0	P I	P II	E	BM	F
12	0 7	0 9	1 1	0 3	0 3	0 8	0 3	0 5	0 7	0 4	0 4	0 4
14	3 6	3 9	4 0	3 2	3 6	4 2	2 9	2 1	2 3	3 2	3 1	3 0
15	0 8	1 3	1 3	0 7	0 5	1 0	0 7	0 7	0 7	0 4	0 3	0 3
16 0	22 2	21 0	24 6	21 6	23 4	24 8	25 8	22 6	25 3	25 6	26 7	26 9
16 1	4 4	3 7	4 0	4 5	3 1	4 4	2 2	1 7	2 3	4 1	3 3	3 7
17	1 2	1 4	1 3	1 1	0 7	1 3	1 6	1 9	1 5	0 3	0 6	0 7
18 0	6 7	5 4	5 [illegible]	6 2	7 4	6 1	12 9	12 4	11 9	8 2	8 5	7 7
18 1	33 8	31 9	28 4	45 4	45 2	37 5	28 4	31 5	28 7	40 8	40 8	40 3
18 2	12 3	11 5	12 7	10 9	11 2	8 9	9 9	10 5	13 3	12 0	11 5	12 0
19	0 2	0 4	0 2	0 4	0 3	0 4	0 3	0 4	0 2	0 2	0 1	0 1
20 0	0 4	0 5	0 4	0 3	0 3	0 4	0 8	0 4	0 3	0 3	0 4	0 3
20 1	1 5	1 7	1 1	1 5	1 2	1 2	1 5	1 3	1 1	1 3	1 0	1 1
20*p*	7 3	10 3	7 3	1 9	0 6	4 7	6 8	7 3	5 9	1 9	0 8	1 1
21	0	0	0	0	0	0	0	0	0	0	0	0
22 0	2 4	2 9	5 6	1 7	1 7	3 3	3 2	4 2	3 4	1 2	2 0	1 8
22 1	0 6	1 0	0 9	0 4	0 3	0 5	0 6	0 4	0 3	0 2	0 3	0 3
22*p*	1 9	2 2	1 6	0 2	0 2	0 5	2 1	2 1	2 1	0 3	0 2	0 3

	F5						D C1				
	P 0	P I	P II	E	BM	F	P 0	P I	P II	BM	F
12	0 9	0 8	1 0	0 2	0 4	1 0	0 2	0 3	0 3	0	0
14	2 3	2 2	2 1	1 9	2 1	2 2	1 [illegible]	1 4	2 9	3 0	1 6
15	0 6	0 8	1 5	0 4	0 5	0 6	1 2	1 7	1 0	0	0 7
16 0	25 4	24 1	21 8	23 3	23 4	23 2	24 5	23 4	25 6	25 4	25 7
16 1	1 5	1 7	1 4	2 5	2 4	2 5	1 3	1 4	2 1	2 7	1 3
17	1 3	1 2	2 1	0 8	1 0	1 5	2 1	3 2	2 0	1 4	1 7
18 0	10 8	11 4	10 5	8 5	8 2	8 6	12 8	11 7	13 0	8 6	12 5
18 1	36 6	35 9	27 1	50 3	49 7	46 3	40 4	38 2	40 5	48 5	41 1
18 2	7 4	8 6	8 3	6 8	6 0	6 2	6 0	6 4	4 3	7 1	[illegible] 4
19	0 2	0 4	0 3	0 3	0 2	0 3	0 5	1 1	0 6	0 2	0 8
20 0	0 6	0 6	0 9	0 5	0 5	0 5	0 4	1 3	0 5	0 2	1 1
20 1	1 5	1 6	1 2	1 9	1 7	1 6	1 0	1 2	0 9	1 1	0 8
20*p*	3 5	4 5	6 9	0 4	1 3	0 5	4 3	4 4	3 2	0 3	2 7
21	0	0	0	0	0	0	0	0	0	0	0
22 0	2 9	2 9	7 9	1 4	1 4	3 3	1 8	2 8	2 0	1 0	3 6
22 1	2 4	2 3	5 0	1 1	1 0	1 5	0 5	0 1	0 1	0 3	0 1
22*p*	2 1	1 1	2 0	0 2	0 2	0 2	1 4	1 4	1 0	0 2	0 9

Table V The correlations in the fatty acid percentages in the triglycerides emboli vs plasma and emboli vs bone marrow in dogs D1–D3 The correlations were compared by Snedecor's F test applied to the residual variances (s^2_{yx}) "*" indicates that the relationship between emboli vs bone marrow is closer ($p<$ 0 01) than emboli vs both plasma I and II

	Emboli—Plasma I		Emboli—Plasma II		Emboli—Bone marrow	
Dog	r	s_{yx}	r	s_{yx}	r	s_{yx}
D1	0 96	3 6	0 98	2 3	1 00	0 5*
D2	0 94	4 2	0 92	4 8	1 00	0 7*
D3	0 99	2 1	0 99	1 7	0 99	1 3

Table VI The correlations in the fatty acid percentages in the triglycerides emboli vs plasma and emboli vs bone marrow in dogs E1–E5 The correlations were compared by Snedecor's F test applied to the residual variances (s^2_{yx}) "*" indicates that the relationship between emboli vs bone marrow is closer ($p<$ 0 01) than emboli vs both plasma I and II

	Emboli—Plasma O		Emboli—Plasma I		Emboli—Plasma II		Emboli—Bone marrow	
Dog	r	s_{yx}	r	s_{yx}	r	s_{yx}	r	s_{yx}
E1	0 97	3 0	0 97	2 9	0 96	3 5	0 99	1 4*
E2	0 99	1 5	0 99	1 9	0 97	2 9	1 00	0 4*
E3	0 98	2 5	0 96	3 3	0 94	4 2	1 00	0 7*
E4	0 96	3 3	0 97	2 7	0 96	3 0	1 00	0 4*
E5	0 98	2 8	0 98	2 9	0 92	5 1	1 00	0 3*

Table VII The fatty acid composition of arterial FFA and of emboli (E) and bone marrow (BM) triglycerides in 4 traumatized dogs (A4 B1 C1 and D3) and in 3 controls (DC1–DC3) In dog B1 and C1 plasma samples (P0) were taken before the administration of coconut butter (B1) or tristearate (C1) In all dogs plasma samples were taken before the fracture or corresponding time (PI) and before the perfusion (PII) In dog D3 and DC1–DC3 norepinephrine was infused during the experiments

	A4				B1				C1			
Fatty acid	P I FFA	P II FFA	F TG	BM TG	P 0 FFA	P I FFA	E TG	BM TG	P 0 FFA	P I FFA	E TG	BM TG
12	0 4	0 3	2 2	0 8	0	0 7	0 2	0 4	0	0 4	0	0 3
14	1 1	1 3	5 0	6 9	1 6	2 9	2 4	3 0	1 6	2 5	3 0	3 4
15	0 7	0 5	1 2	0 7	0 8	1 0	0 5	0 3	0 8	0 8	0 2	0
16 0	25 1	29 7	23 1	25 0	29 1	31 3	21 1	19 6	24 7	28 3	25 3	25 5
16 1	5 6	4 3	5 6	4 9	2 5	2 1	5 3	4 1	3 3	1 9	3 2	2 9
17	2 0	2 1	1 8	1 7	2 5	3 1	1 1	1 2	2 4	1 6	0 7	0 5
18 0	11 1	11 1	7 6	7 5	16 6	18 2	9 0	10 2	11 9	21 2	7 1	7 9
18 1	40 9	36 7	43 5	42 5	27 9	26 6	50 0	52 2	37 5	29 9	47 9	47 3
18 2	7 7	8 4	6 7	7 4	3 8	4 4	7 7	7 4	7 2	5 9	7 3	7 9
19	0 7	0 6	0 7	0 3	0 3	0 6	0 3	0 1	1 4	0 6	0 3	0 2
20 0	0 4	0 2	0 4	0 3	0 3	0 6	0 5	0 2	2 0	1 0	0 4	0 4
20 1	0 1	0 2	1 7	1 2	0 4	0 6	1 3	1 0	1 2	0 9	1 2	1 1
20*p*	0 4	2 2	0 6	0 4	1 8	1 1	0 2	0 1	1 1	1 0	2 6	1 4
21	0 7	0 1	0	0	0 4	1 4	0	0	0	0	0	0
22 0	0 4	0 2	0	0	0 5	0 6	0 1	0	3 7	2 3	1 0	0 8
22 1	0 7	0 7	0 5	0 4	3 8	2 2	0 3	0 1	1 0	0 8	0 1	0 2
22*p*	2 1	1 4	0 1	0 2	3 1	1 9	0 1	0 1	0 2	0 9	0 1	0 2

REFERENCES

1 Carlson L A & Bally P R Inhibition of lipid mobilization In Handbook of Physiology Section 5 Chapter 57 Waverly Press Baltimore USA 1965

2 Carlson L A Boberg J & Hogstedt B Some physiological and clinical implications of lipid mobilization from adipose tissue In Handbook of Physiology Section 5 Chapter 63 Waverly Press Baltimore USA 1965

3 Carlson L A Liljedahl S O & Wirsen C Blood and tissue changes in the dog during and after excessive free fatty acid mobilization Acta med scand 178 81 1965

4 Duncombe W G The colorimetric micro determination of long chain fatty acids Biochem J 88 7 1963

5 Gustafson A & Kerstell J Serum lipoprotein pattern in fat embolism in the dog Acta med scand Suppl 499 19 1969

6 Hallgren B Kerstell J Rudenstam C M & Svanborg A A method for the isolation and chemical analysis of pulmonary fat emboli Acta chir scand 132 613 1966

7 Hallgren B Kerstell J Rudenstam C M & Svanborg A The chemical composition of the fat emboli in the post alimentary dog Acta med scand Suppl 499 25 1969

8 Kerstell J The incorporation of labelled chylo microns in the fat emboli in the post alimentary dog Acta med scand Suppl 499 33 1969

9 Kerstell J Studies on the pathogenesis of post traumatic fat embolism Thesis Flanders Bok tryckeri Goteborg Sweden 1969

10 Kerstell J The chemical composition of fat emboli in dogs with extirpated liver and intestines Acta med scand Suppl 499 37 1969

11 Kerstell J Hallgren B Rudenstam C M & Svanborg A The chemical composition of the fat emboli in the post absorptive dog Acta med scand Suppl 499 3 1969

12 Liljedahl S O & Westermark L Aetiology and treatment of fat embolism Acta anaesth scand 11 177 1967

GÖTEBORG 1969
ELANDERS BOKTRYCKERI AKTIEBOLAG

Acta Medica Scandinavica

Supplementum 500

Glucocorticoids in Internal Medicine

A Symposium on Systemic Therapy other than Substitution Therapy

REDIGENDA CURAVIT
IB LORENZEN

Distributed by The Almqvist & Wirsell Periodicals Company Stockholm, Sweden

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv*, was founded in 1869 by Professor Axel Key, MD In 1901 (from volume 34) this journal was devided into a medical and a surgical section *Since 1919 (from volume 52) the medical section has been* published under the name of *Acta Medica Scandinavica*

Acta Medica Scandinavica
publishes papers on general medicine mainly from Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Short preliminary reports (not exceeding two pages) are published promptly The papers are published in English, French or German Acta Medica Scandinavica is published on a non profit basis

Subscriptions
to Acta Medica Scandinavica (two volumes of six numbers each a year) include free supplements to the current volumes During the past few years an average number of 17 supplements has been issued per annum

Subscription Rates
Per annum – two volumes
In Denmark, Finland, Iceland, Norway, Sweden and the Netherlands Sw kr 150, incl postage
Other countries Sw kr 175, incl postage

Chief Editor
Associate Professor Birger Strandell, MD
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden

Editorial Office
Acta Medica Scandinavica
Birger Jarlsgatan 9
S-111 45 Stockholm, Sweden
(All correspondence concerning manuscripts and editorial matters)

Advertising
Medicinska Annonsexpeditionen AB
Biblioteksgatan 11
S-111 46 Stockholm Sweden

Subscription and Distribution
The Almqvist & Wiksell Periodicals Company
Gamla Brogatan 26, Box 62
S 101 20 Stockholm 1, Sweden

Printers
Almqvist & Wiksells Boktryckeri AB
Box 616
S-751 25 Uppsala 1, Sweden

ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 500

GLUCOCORTICOIDS IN INTERNAL MEDICINE

A Symposium on Systemic Therapy other than Substitution Therapy

Held in Fredensborg Denmark
May 24th 1968

REDIGENDA CURAVIT
IB LORENZEN

Copenhagen 1969

Printed in Denmark

BIANCO LUNOS BOGTRYKKERI A/S
COPENHAGEN

CONTENTS

CHAIRMAN	IB LORENZEN M D Chief Physician Medical Department C Gentofte Hospital Hellerup Copenhagen
SPEAKERS	BINDER CHRISTIAN M D Medical Department C Diakonissestiftelsen Copenhagen
	EFFERSOE POUL M D Chief Physician Department for Communicable Diseases Blegdam Hospital Copenhagen
	HVIDBERG EIGILL M D Medical Department A Rigshospitalet University of Copenhagen Copenhagen
	JENSEN HERLUF M D Medical Department P Rigshospitalet University of Copenhagen Copenhagen
	LORENZEN IB M D Chief Physician Medical Department C Gentofte Hospital Hellerup Copenhagen
	QUAADE FLEMMING M D Chief Physician Medical Department B Bispebjerg Hospital Copenhagen
	RIIS POVL M D Head of Medical Department B Gentofte Hospital Hellerup Copenhagen
	SCHWARTZ MICHAEL M D Head of Medical Department F Glostrup Hospital Copenhagen
	VIDEBÆK AAGE M D Professor of Internal Medicine Head of Medical Department C Gentofte Hospital Hellerup Copenhagen
OTHER PARTICIPANTS IN THE DISCUSSIONS	ANDERSEN MOGENS M D Medical Department B Rigshospitalet University of Copenhagen Copenhagen
	ANDERSSON ERIK M D Head of Medical Department County Hospital Fakse
	ANDRESEN PAUL M D Head of Medical Department County Hospital Ribe
	ASBOE HANSEN GUSTAV M D Professor of Dermatology Chairman of Department of Dermatology Rigshospitalet University of Copenhagen Copenhagen
	BENNIKE TORBJØRN M D Head of Medical Department County Hospital Aalborg
	BRUUN EGON M D Head of Clinic for Allergic Diseases Rigshospitalet University of Copenhagen Copenhagen
	HARVALD BENT M D Chief Physician Medical Department C Bispebjerg Hospital Copenhagen

HILDEN, TAGE, M D, Head of Medical Department C Diakonissestiftelsen Copenhagen

IVERSEN KURT, M D Professor of Internal Medicine Head of Medical Department II, Municipal Hospital Copenhagen

KALBAK, KAJ M D Head of Hospital for Rheumatic Diseases Graasten

KIRKETERP POUL M D Head of Medical Department Municipal Hospital Horsens

KJERULF, KAJ M D, Head of Medical Department, Blegdam Hospital Copenhagen

LOU, HANS, M D, Head of Medical Department, County Hospital, Kolding

MOSBECH JOHANNES M D Head of Medical Department, St Elisabeth Hospital Copenhagen

NISSEN NIS I M D Head of Medical Department Finsensinstituttet Copenhagen

OLESEN KNUD M D, Chief Physician Medical Department B Rigshospitalet, University of Copenhagen, Copenhagen

ROSSEL IB M D, Department of Physical Medicine and Rheumatology, Rigshospitalet University of Copenhagen Copenhagen

SPRECHLER MOGENS M D Head of Surgical Department F, Bispebjerg Hospital Copenhagen

WITH TORBEN K M D, Consultant Chemical Pathologist Head of Department of Clinical Chemistry, County Hospital Svendborg

WORNING HELGE M D Chief Physician Medical Department F Glostrup Hospital Copenhagen

PREFACE

It is now 20 years since Hench and his colleagues demonstrated the dramatic clinical effect of cortisone in rheumatoid arthritis and thereby initiated the clinical application of the glucocorticoids other than as substitution therapy Since that time there has been an enormous expansion of the range of indications The immeditate effect in other disorders is often just as striking as that in rheumatoid arthritis However two circumstances have led to a diminution of the enthusiasm which followed the first therapeutic results The first is that the effect of the glucocorticoids would seem primarily to be symptomatic and the second that when used in pharmacological doses the glucocorticoids can under certain circumstances give rise to serious side effects It is especially this latter finding which has made it necessary to submit the therapy to critical revision once in a while Are the glucocorticoids capable of changing the course of the illness its duration or its prognosis or have they exclusively a symptomatic effect? Do the glucocorticoids in some disorders even lead to a deterioration in the prognosis? Should the indications for therapy be narrowed and the use of the glucocorticoids in certain diseases perhaps altogether abandoned despite a satisfactory symptomatic effect?

It is some of these questions that we hope to answer during the symposium On the basis of published results of treatment we will attempt to make a critical evaluation of the therapeutic value of the glucocorticoids in some of the commonest and apparently most vital ranges of indication

This symposium is the fifth which has been arranged by *Merck Sharp & Dohme* for Danish specialists in Internal Medicine Our thanks are also due to *Merck Sharp & Dohme* for financing the publication of the proceedings of the symposium

Ib Lorenzen

Acta med scand Suppl 500 pp 9-16 1969

THE PHYSIOLOGY AND PHARMACOLOGY OF THE GLUCOCORTICOIDS

by
Christian Binder

The term glucocorticoids came into general use in about 1950 It referred to the powerful action of certain of the adrenal corticosteroids on the carbo hydrate metabolism As will become apparent the glucocorticoids are also of importance in the meta bolism of protein fat water and electrolytes whilst their action on a number of physiological and patho logical processes cannot be referred to any known metabolic process In the following the term gluco corticoids is used of steroids—either naturally occurring or synthetic—which in their effect and probable mode of action resemble cortisol

Today a great deal is known about the metabolic and biochemical changes which occur at various intervals after the administration of glucocorticoids On the other hand very little is known about the way *in which the glucocorticoids exert their effect at* cellular and molecular levels

In this review the main emphasis will be laid on those actions of the glucocorticoids which are mani fest when the hormones are administered clinically In conclusion a characteristic of the available gluco corticoids is included

THE METABOLIC ACTIONS OF THE GLUCOCORTICOIDS

Carbohydrate metabolism

In adrenal failure and after adrenalectomy fasting or the ingestion of carbohydrate poor diet leads to hypoglycaemia This is corrected on the administra tion of glucocorticoids At the same time there is a raised excretion of nitrogen The administration of pharmacological doses of glucocorticoid leads to hyperglycaemia glycosuria diabetic reaction to the administration of glucose and increased deposition of glucose in the liver

An increase in the blood sugar concentration may be due to an increased glucose production in the liver or a reduction in the utilization of glucose The ability of the glucocorticoids to increase glyconeo genesis is well documented (13 16 31 43) It would appear that the increased glucose production is due in part to the fact that the glucocorticoids have an inhibitory effect on the conversion of pyruvic acid to acetyl-coenzyme A (39) This leads to an accumula tion of pyruvic acid which again leads to the resyn thesis of glucose The glucocorticoids would seem to have no effect on the utilization of glucose when they are administered in physiological doses After larger doses there is in contrast a reduction in utili zation (13) It has not been clarified whether this is due to the steroid itself or whether it is due to a relative lack of insulin

The increased deposition of glycogen in the liver is *observed three to twenty four hours after the admin* istration of glucocorticoid (3) this is considerably later than the increase in blood sugar concentration On the other hand it shows a close correlation with the increased excretion of nitrogen in the urine

Protein metabolism

The catabolic or anti anabolic effect of the glucocor ticoids is best demonstrated by patients who either produce too much hydrocortisone or who have received excessive amounts of glucocorticoids The negative nitrogen balance is associated with inhibi tion of growth osteoporosis muscular atrophy reduction in skin thickness and a reduction in the amount of lymphoid tissue On the other hand the protein content of the liver is increased The cata bolic action of the glucocorticoids may be a result of a demonstrated (3) increase in the uptake and breakdown of amino acids in the liver (33 35)

A large part of the amino acids is deaminated and converted to glucose but part is also utilized for the synthesis of protein especially ribonucleic acids and enzymes The amounts in the liver of a number of

the enzymes which are involved in carbohydrate and protein metabolism are increased during glucocorticoid therapy (28) With a few exceptions the increases are secondary to the changes in carbohydrate and protein metabolism which have been mentioned In this connection it should be brought to mind that the metabolic changes caused by the glucocorticoids are counteracted by insulin (1) Adrenalectomy leads to *improvement in an existing diabetes mellitus* and steroid-diabetes is a well recognised phenomenon The glucocorticoids increase the peripheral breakdown of proteins In the liver the glucocorticoids enhance the synthesis of enzyme protein whilst this process is inhibited by insulin (2 41 43)

Fat metabolism

In patients who produce or receive excessive amounts of glucocorticoid there is an increased deposition of subcutaneous fat on the trunk at the back of the neck and in the face at the expense of the subcutaneous fat in the limbs Glucocorticoids are essential for the mobilization of fatty acids from the fat depots (20) It has thus been demonstrated that adrenaline and other lipolytic agents are ineffective in adrenalectomized animals (18 38) The full role of the glucocorticoids in fat metabolism is otherwise unclarified

Water and electrolyte metabolism

Sodium retention hypokaliaemia and water retention are dreaded complications of glucocorticoïd therapy As will be apparent later there are now a number of glucocorticoids available which have reduced or a complete absence of mineralocorticoid action There is however one action which is independent of the mineralocorticoid action This is the reaction to a water load which is definitely related to the glucocorticoid action

The lack of diuretic response to a water load in patients with adrenal cortical failure can only be corrected by the administration of glucocorticoid The mineralocorticoids have no effect This finding has been utilized in the diagnosis of adrenal cortical failure When administered in pharmacological doses to normal individuals the glucocorticoids lead to an increased diuresis (34) The physiological background of this action has not been clarified but it is natural to imagine that it is due to a direct effect on the kidneys although no evidence has been put forward to confirm this It is not possible to substantiate the theory that cortisol exerts an antagonistic action on the antidiuretic hormone (ADH) in the kidneys as the sensitivity of the kidneys to vasopressin is not affected by the administration of cortisol (27 29)

A number of observations would suggest that the changes in volume and osmotic regulation are part of the processes which condition the action of the glucocorticoids on the water balance Thus patients *with adrenal cortical failure show a normal diuretic* response to a water load without the administration of glucocorticoids if the reduced extracellular volume in the patient has been restored to normal beforehand (17) It has also been demonstrated that the administration of glucocorticoids to normal individuals raises the osmotic threshold for the release of ADH in response to an osmotic stimulus (4) These observations are probably of no importance in the pharmacological application of the glucocorticoids but they illustrate the fact that despite the many observations of the actions of glucocorticoids it is still not possible to draw any definite conclusions about their basis physiological importance for the function of the intact organism

Calcium metabolism

Osteoporosis and spontaneous fracture are other undesirable actions of glucocorticoid therapy The glucocorticoids inhibit the formation of the bone matrix and the proliferation of epiphysial cartilage in rats (7) In children who are receiving glucocorticoid therapy there may be severe retardation of growth The concentration of calcium in the serum of patients with spontaneous fractures is normal and the concentration of alkaline phosphatases is likewise not increased (36)

The administration of glucocorticoid to rats which have undergone parathyroidectomy inhibits the absorption of calcium from the intestine (12) and the hypocalcaemia which occurs in patients with hypoparathyroidism deteriorates on the administration of glucocorticoids (14) It must be assumed that in the intact organism this action on the calcium metabolism is counteracted by a hypersecretion of parathormone This in itself would give rise to an increased resorption of bone

In the hypercalcaemia which is seen in sarcoidosis (22) multiple myelomatosis (32) and hypervitaminosis D (40) the administration of glucocorticoids reduces the calcium concentration in the serum to normal by inhibiting the absorption of calcium from the intestine (22)

The action of the glucocorticoids on inflammatory processes including the effect on vasomotor tone and capillary permeability will like the effect on immune reactions and on the haemopoietic system be described elsewhere in this symposium

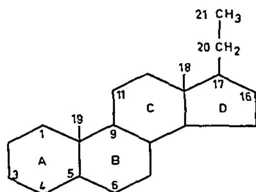
As a conclusion to the first half of this paper a single observation will be mentioned This may be found to be of importance for an understanding of the pharmacological action of the glucocorticoids The lysosomes are among the many organelles of the cells They are surrounded by a membrane and contain enzymes which could bring about the destruction of the structures of the remainder of the cell The lysosome membrane is destroyed by among other things ischaemia streptolysins endotoxins and vitamin A The glucocorticoids seem to stabilize the lysosome membrane against these influences (42)

GLUCOCORTICOID PREPARATIONS

For about two years after Hench introduced the agent into the therapeutic armamentarium (21) cortisone acetate was the only glucocorticoid available In 1950 cortisol was synthesized this had an anti inflammatory action which was about 25 % greater than that of cortisone It soon became clear that cortisol was the natural glucocorticoid The endeavours during the following years had two leitmotivs the desire to synthesize a more potent glucocorticoid and the desire to synthesize a steroid with a specific anti inflammatory action The first of these has been fulfilled as today glucocorticoids are available which have an anti inflammatory effect which is 2 500 times as great as that of cortisol but the second aim has not yet been fulfilled It rapidly became clear that it was possible to alter the steroid molecule such that the glucocorticoid or mineralocorticoid activity could be intensified at will but the increase in anti inflammatory action was associated with an intensification of the other glucocorticoid actions

Today there are a number of different glucocorticoid preparations available all of which are claimed to be the most effective A review of the structural changes in the steroid molecule and the relation between these and the effects which are observed when the agent is administered will presumably facilitate the evaluation of the preparations which are and in future will be offered to us

All the adrenal cortical steroids can be derived from pregnane the structural formula of which is shown in figure 1 The skeleton of the steroid molecule consists of three six membered rings A B and C and a five membered ring D with methyl groups at positions C_{10} and C_{13} The glucocorticoids furthermore have a side-chain of 2 C atoms at C_{17} The numeration of the carbon atoms may be seen from the figure Double links are indicated by the suffix '-en(e) A hydroxyl group is indicated by dihydroxy and trihydroxy / -diol and triol Carbonyl groups have the prefix oxo (or keto-) and one or -dione as suffix The position of the group is indicated by naming the number of the



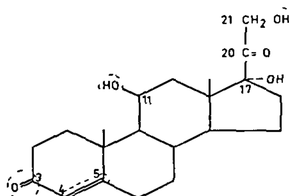
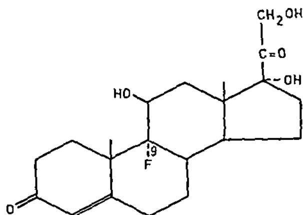


Fig 1



9α - Fluorcortisol

Fig 2

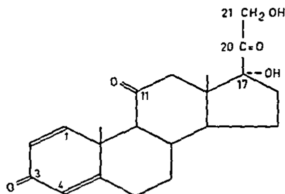
carbon atom Where there is a possibility of isomerism α indicates a transposition which is shown in the formula as a stippled line β indicates that the group is in the cis position and is shown by a solid line Cortisol which is shown on the right in figure 1 is thus 11β, 17α 21 trihydroxy Δ^4 pregnen 3 20 dione Δ^4 refers to the double bond from C_4 to C_5

The groups which are essential to the glucocorticoid action are marked with a ring If even one of these is altered, the glucocorticoid action of the steroid concerned disappears

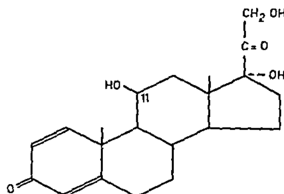
The first advance in the synthesis of new analogues was made in 1953 with the introduction of halogen atoms in α position at C_9 (15) This led to an intensification of several of the properties of cortisol (figure 2) These were the fluor substituted preparations which had the most powerful anti inflammatory effect measured by the effect on patients with rheumatoid arthritis they were 10 times as active as cortisol However, it rapidly became apparent that the *mineralocorticoid action was increased* to an even greater extent than the glucocorticoid action The mineralocorticoid effect is considered to be 600 times that of cortisol Today these are the most potent synthetic mineralocorticoids available commercially

The next important step was the synthesis of prednisone (23) the structural formula of which is shown in figure 3 which also shows the formula of prednisolone It is the latter which is the active glucocorticoid but as hydroxylation at C_{11} takes place freely *in vivo* the two substances may be substituted one for the other This is in contrast to what is the case with cortisone and cortisol Because of the rapid inactivation of cortisone in the liver a variable part at most 80 % of any given dose of cortisone will be converted to the biologically active cortisol Cortisol should therefore be used in preference to cortisone (25) Table I shows the amounts of the various glucocorticoids which exert approximately the same anti inflammatory action

In 1956 a methyl group was inserted in the α position at C_6 (24) (figure 4) This intensified the glucocorticoid action of both cortisol and prednisolone by a factor of three to four However, there was a corresponding increase in the undesirable effects and



Prednisone



Prednisolone

Fig 3

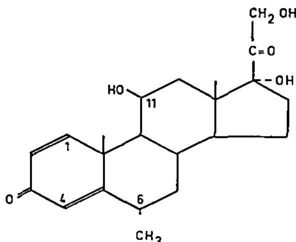
Table 1 Amounts of the various glucocorticoids which exert approximately equivalent effects in man

Glucocorticoid	Dose (mg)
Cortisol	20
Cortisone	25
Prednisolone	4–5
Prednisone	4–5
Methylprednisolone	3–4
Fluoxiprednisolone	3–4
Fluormethylprednisolone	0 50–0 75
Flubenisolone	0 50–0 75

these compounds have no advantages or disadvantages as compared with prednisone and prednisolone (11)

Substitution at C_{16} has comprised hydroxylation and methylation If a hydroxy group is introduced in the α position in cortisol or prednisolone the glucocorticoid action of these compounds is reduced (8) If on the other hand a hydroxy group is introduced at C_{16} in 9α fluoro-prednisolone this gives a very potent glucocorticoid fluoxyprednisolone (figure 5) In addition to its powerful anti inflammatory effect this substance has proved to have a number of undesirable actions of which the most prominent are the production of myopathy anorexia and loss of weight (10)

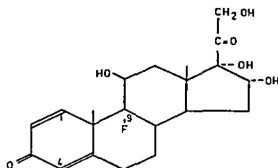
Introduction of a methyl group at C_{16} does not alter the glucocorticoid action unless there is already a fluorine atom at C_9 If this is the case then it makes no difference whether the methyl group is in the α or the β-position (8) The two preparations are called fluormethylprednisolone (figure 5) and flubenisolone Neither of these preparations has any advantages over prednisone and prednisolone in



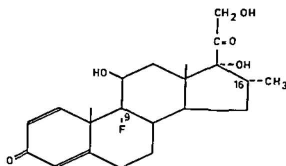
6 α - methylprednisolone

(Methylprednisolone USP)

Fig 4



9 α - fluor-
16 α - hydroxyprednisolone
(Fluoxiprednisolonum NFN)
(Triamcinolone USP)



9 α - fluor-
16 α - methylprednisolone
(Fluormethylprednisolonum NFN)
(Dexametasone USP)

Fig 5

long term therapy Dexamethasone stimulates the appetite and may be used when such an action is considered desirable It has a powerful suppressive effect on the hypothalamic pituitary regulation of ACTH secretion and is therefore used in diagnostic investigations in which a suppression of the secretion of ACTH is required

If a fluorine atom is introduced at C_6 in prednisolone there is an increase in the anti inflammatory effect by twice or three times whilst at the same time there is less tendency for the patient to put on weight Similarly, the Cushingoid appearance in patients treated with prednisone vanished when they were transferred to equipotent doses of the former agent (10) Paramethasone has in addition to a fluorine atom at C_6 a methyl group at C_{16} Its action should correspond to that of 6α fluor prednisolone although in contrast to the latter it leads to an increase in weight an action which must presumably be ascribed to the methyl group at C_{16} (10)

The conclusion of a review of the literature dealing with the relation between the actions of the synthetic glucocorticoids is that as yet no steroid has been synthesized which is superior in all respects to prednisone and prednisolone In special cases a change from these two preparations to one of the remainder may be indicated especially where there is a supplementary requirement of either increase or reduction in weight

DISCUSSION

Dr Andresen With regard to the relation cortisone versus cortisol i e the keto group versus the hydroxyl group it is interesting that the most marked effect is obtained by the administration of cortisol Are there corresponding findings with prednisone and prednisolone? In a recent publication ("Endocrinology last autumn) the effects of prednisone and prednisolone were studied Prednisolone had a more marked effect on DNA-synthesis and protein synthesis than prednisone

Dr Binder It is probably advisable to be cautious in drawing conclusions on the basis of the effects of the steroids on tissue and cell cultures but we know that the conversion of prednisone to prednisolone can take place in the cell Investigations have been carried out in man on the conversion between cortisone and cortisol and prednisone and prednisolone Cortisone was hydrated to cortisol in the liver to an extent corresponding to at most 80 / of the dose administered whilst this difference did not seem to exist between prednisone and prednisolone

Dr Loren en This brings us to the problem of the relative activities of the different glucocorticoids and the question of the relation between the clinical effect and the metabolism of the glucocorticoids in the tissues Sometimes quite small doses of glucocorticoids are able to suppress the activity of disease doses which appear to be less than the endogenous cortisol production

Dr Hedberg In the course of the past few years an enormous body of details has accumulated about the effect of the glucocorticoids on a large number of processes However as yet this has had no noticeable effect on our clinical practice It is apparent from what Binder has said that by means of changes in the hydrocortisone molecule first prednisolone and later a long series of other compounds have been produced which mg for mg are more potent whilst at the same time the mineralocorticoid actions have disappeared On the other hand a large number of the other actions including undesirable effects are unchanged Similarly there is almost no change in the qualitative influence on many of the more basic processes processes which are more or less correctly considered to be related to the anti inflammatory properties These include various metabolic processes enzymatic systems and the effects on the subcellular substances and structures and the associated changes in the capillary and cell membrane permeability antibody production etc (30 44) In recent years it has been demonstrated that the structural changes in the glucocorticoid molecule can be correlated to changes in the metabolism of the compounds their affinity to specific substances especially protein binding their ability to penetrate and other biochemical parameters There would seem to be both certain quantitative and qualitative differences in this respect and it is these which give rise to the practical therapeutic consequences

The changes suggested would seem to influence the local concentration of the agents among other things It does not matter which of the theories concerning the mechanism of anti inflammatory action one accepts the effect of the compounds must in addition to their intrinsic activity be determined by their concentration at the site of action and this is of course at the subcellular level

Metabolic stability thus plays an important part and in this respect there may be considerable differences between the individual glucocorticoids These differences cannot provide the complete explanation for the differences between the anti inflammatory effects but they can contribute to such an explanation

It is thus known that in the body methyl prednisolone is metabolized more slowly than prednisolone and that the latter is again metabolized more slowly than hydrocortisone An increase in the number of substitutions as Binder has demonstrated will generally increase the metabolic stability of the compound such that it will presumably remain for a longer period in the area on which it acts The relation between the two types of metabolism will also be altered by this such that relatively less is metabolized or inactivated in the liver and relatively more is broken down in the peripheral tissue (30 37) Another factor which is to a high degree co-determinant for the active concentration is the protein binding The specific binding to transcortin and the more non-specific binding to albumin is altered it seems to decrease with the number of substitutions (9 37) or with the increasing degree of complexity of the substituents This has been demonstrated both *in vitro* and *in vivo* Particular attention has been paid to the plasma protein binding but in fact there should be far more interest in

the binding which occurs with various more or less specific substances outside the vascular system The necessary active concentration of the glucocorticoids at the cellular level is naturally completely unknown Other related clinical pharmacological problems are otherwise completely unsolved We know nothing about the relation of the dose given to the individual patient to the plasma concentration obtained and the relation of this to the incidence and nature of the undesirable effects

It has also become apparent that both the rheumatic diseases in themselves and the treatment administered are capable of changing the metabolic conditions and other physical-chemical characteristics It has thus been demonstrated that the metabolism of hydrocortisone and prednisone is altered in patients with rheumatoid arthritis and perhaps also in other patients This had previously been disputed (5 6 26) Furthermore it has been found that in patients with rheumatoid arthritis there is a change in the metabolic products of exogenously administered hydrocortisone after long term treatment with prednisone (19) This seems to imply that the metabolic pathways are altered by antecedent treatment with other glucocorticoids The phenomena of enzyme induction or enzyme inhibition facilitated by the glucocorticoids must be borne in mind in this connection

Dr Lorenzen Chemical differences between the steroids administered and the endogenous glucocorticoids and changes in the metabolism of the steroids as a result of disease may therefore provide an explanation for the finding that it is occasionally possible to inhibit the activity of a disease with doses which are considerably lower than the endogenous glucocorticoid production

REFERENCES

1 Ashmore J The effect of glucocorticoids on insulin action Diabetes 13 134-354 1964
2 Ashmore J Hastings A B Nesbett F B & Renold A E Studies on carbohydrate metabolism in rat liver slices J Biol Chem 218 77-88 1956
3 Ashmore J & Morgan D Metabolic effects of adrenal glucocorticoid hormones in Eisenstein A B The adrenal cortex by 26 authors London p 249 267 1967
4 Aubry F M Mankin H R Moses A M & Streeton D H P Measurement of the osmotic threshold for vasopressin release in human subjects and its modification by cortisol J clin Endocr 25 1481-1492 1965
5 Bailey E Greaves M S & West H F Hydrocortisone metabolism in rheumatoid arthritis Lancet II 431-434 1967
6 Bailey E & West H F Prednisolone metabolism and rheumatoid arthritis Lancet II 1231-32 1967
7 Bernick S & Ershoff B H Histochemical study of bone in cortisone treated rats Endocrinology 72 231-236 1963
8 Bernstein S The chemistry and biological activities of 16-hydroxylated steroids Recent Progr Hormone Res 14 1-30 1958
9 Blecher M Serum protein steroid hormone interaction Endocrinology 79 541-546 1966
10 Boland E W Chemical comparison of the newer anti inflammatory corticosteroids Ann rheum Dis 21 176-191 1962
11 Boland E W & Liddle G W Metabolic and antirheumatic activities of 6α methyl prednisolone (medrol) Ann rheum Dis 16 297-306 1957
12 Clark I Geoffroy R F & Bowers W Effects of adrenal corticosteroids on calcium metabolism Endocrinology 64 849-856 1959
13 DeBodo R C & Altzuler N Insulin hypersensitivity and physiological insulin antagonists Physiol Rev 38 389-445 1958
14 Eliel L P Thomsen C & Chanes R Antagonism between parathyroid extract and adrenal corticosteroids in man J clin Endocr 25 457-464 1965
15 Fried J & Sabo E F Synthesis of 17α hydroxy corticosterone and its 9α halo derivatives from 11-epi 17α hydroxycorticosterone J Amer chem Soc 76 2273-2275 1953
16 Froesch E R Ashmore J & Renold A E Comparison of renal and hepatic effects of fasting, cortisone administration and glucose infusion in normal and adrenalectomized rats Endocrinology 62 614-629 1958
17 Gill J R Gann D S & Bartter F C Restoration of water diuresis in Addisonian patients by expansion of the volume of extracellular fluid J clin Invest 41 1078-1085 1962
18 Goodman H M & Knobel E *Some endocrine factors* in regulation of fatty acid mobilization during fasting. Amer J Physiol 201 1-3 1961
19 Grayzel E F & Longson D Cortisol tolerance in rheumatic diseases Lancet II 66-68 1964
20 Havel R J & Goldfien A Adrenal and FFA mobilization J Lipid Res 1 102-108 1959
21 Hench P S Kendall E C Slocumb C H & Polley H F The effect of a hormone of the adrenal cortex and of pituitary adrenocorticotropic hormones on rheumatoid arthritis Proc Mayo Clin 24 181-197 1949
22 Henneman P H Dempsey E F Caroll L & Albright F The cause of hypercalcuria in sarcoidosis and its treatment with cortisone and sodium phytate J clin Invest 35 1229 1242 1956
23 Herzog H L Nobile A Tolksdorf S Clearney W Hersberg E B & Perlman P L New anti arthritic steroids Science 121 176 1955
24 Hogg J A Lincoln F H Jackson R W & Scheider W P The adrenal hormones and related compounds J Amer chem Soc 77 6401-6402 1955
25 Jenkin J S & Sampson P A Conversion of cortisone to cortisol and prednisone to prednisolone Brit med J I 205-207 1967
26 Kelley V C & Ely R S Production and metabolism of adrenocorticosteroids in connective tissue Ann N Y Acad Sci 86 1115-1128 1960
27 Kleeman C R Czaczkes J W & Cutler K Mechanisms of impaired water excretion in adrenal and pituitary insufficiency J clin Invest 43 1641-1648 1964
28 Knox W E Auerbach V H & Lin E C E Enzymatic and metabolic adaptations in animals Physiol Rev 49 164-254 1956
29 *Lindeman R D Carmer van Buren H* & Raisz, L G Effects of steroids on water diuresis and vasopression sensitivity J clin Invest 40 152-158 1961

30 London D R The cort costero ds The Pract t oner 200 113 120 1968
31 Long C N H Katz n B & Fry E G The adrenal cortex and carbohydrate metabol sm Endocr nology 26 309 344 1940
32 Merigan T C & Hayes R E Treatment of hypercal cem a n mult ple myeloma Arch ntern Med 107 389 394 1961
33 Noall M W R ggs T R Walker L M & Chr sten sen H U Endocr ne control of am noac d transfer Sc ence 126 1002 1005 1957
34 Raisz L G McNeely W F Saxon L & Rosen baum J D The effec s of cort sone and hydrocort sone on water d ures s and renal funct on n man J cl n Invest 36 767 779 1957
35 Roberts N R Rosen F Budn ck L E & N chol C A An enzymat c bas s for the gluconeogenet c act on of hyd ocor sone Sc en e 127 287 288 1958
36 Rosenberg E F Rheumato d arthr t s Osteoporos s *and fractures related to stero d therapy Acta med* scand Suppl 341 211 2?4 1958
37 Sarett, L H & Patchett A A The effects of structural alterat on on the ant inflammatory propert es on hydro cort sone Arzne m ttel 5 13 149 196?
38 Shafrir E & S e nberg D The essent al role of the adrenal cortex in the response of plasma free fatty ac ds cholesterol phosphol p ds to ep nephr ne njec t on J cl n Invest 39 310-319 1960
39 Thorn G E Renold A E & Winegrad A I Some effects of adrenal cort cal stero ds on intermed ary metabol sm Br t med J II 1009 1017 1957
40 Verner J V jr Engel F L & McPherson H T Vitam n D intox cat on report of two cases treated w th cort one Ann intern Med 48 765 773 1958
41 Weber G Sn gkal R L & Sr vastava S K Insul n suppressor of b osynthes s of hepat c gluconeogen c en zymes Proc nat Acad S (Wash) 53 96–104 1965
4? We ssman G & Thomas L The effects of cort cos tero ds upon connect ve t ssue and lysosomes Recent Progr Hormone Res 20 215 245 1964
43 Welt I D Stetten D Ingle D J & Morley E H Effect of cort sone upon rates of glucose product on and ox dat on n the rat J b ol Chem 197 57 66 1952
44 *Wh tehouse M W Some b ochem cal* and pharmaco log cal p opert es of ant nflammatory drugs Arzne m ttel 8 32?–419 1965

Acta med scand Suppl 500 pp 17-21, 1969

THE EFFECTS OF THE GLUCOCORTICOIDS ON CONNECTIVE TISSUE

by
Ib Lorenzen

The basis for the widespread clinical use of the corticosteroids outside substitution therapy is their profound influence on the mesenchymal tissues This is due to the fact that a number of the disorders in which treatment with corticosteroids may be considered primarily involve the connective tissue Furthermore the cells of the body are embedded in mesenchymal tissue and the blood vessels are built up of such tissue The mesenchymal tissues are therefore of crucial importance in the normal function of all cells

Our knowledge of the effect of the glucocorticoids on connective tissue is primarily based on animal studies in which unphysiological concentrations of these hormones have been employed This is obviously also of great practical clinical interest but makes no important contribution to our comprehension of the physiological effects of the glucocorticoids on connective tissue

In the clinical use of the glucocorticoids two different sides of their effects on the connective tissue are of interest the influence on the normal connective tissue and the effect on the pathological processes which take place in the tissues primarily the reparative and inflammatory processes

The following account is based on a number of reviews to which the reader is referred for further details The survey is primarily concerned with the loose connective tissue which has been studied most extensively The effects of glucocorticoids on other types of connective tissue are however of a similar nature

The glucocorticoids exert their influence on the connective tissue primarily *via* the mesenchymal cells (2 3 11 12 17) The prototype of the mesenchymal cells is the fibroblast Unphysiological concentrations of glucocorticoids inhibit fibroblast proliferation This is most obvious in granulation tissue in which there is normally vigorous cell proliferation High doses of steroids lead to a reduction in the number of fibroblasts There are furthermore morphological changes in the cells They become more epitheloid vacuoles arise in the cytoplasm and there are changes in the shape of the nucleus occasionally amounting to pyknosis Pinocytosis and mitochondrial movements are abolished High concentrations of glucocorticoids may even lead to cell destruction In contrast to this physiological concentrations would seem to stimulate cell growth The glucocorticoids exhibit a similar inhibitory effect on the mesenchymal cells in bone and cartilage and on the mast cells which are reduced in number and show morphological changes in the nuclei and granules

The influence of the glucocorticoids on the cell metabolism forms the basis for their effect on the extracellular tissue components

Traditionally the extracellular substance in connective tissue is divided into the so-called amorphous intercellular substance and the fibres the majority of which are collagen fibres Our conception of the amorphous intercellular substance has been radically altered during the past decade It has become apparent that the intercellular substance is composed of a network of electrically charged macromolecules Moreover it has been demonstrated that the amorphous intercellular substance is built up of a number of phases of varying composition The most important macromolecules in the amorphous intercellular substance are the mucopolysaccharides in particular the acid mucopolysaccharides which include among others hyaluronic acid and various chondroitin sulphates These compounds seem to occupy a key position in the physiology and pathophysiology of the connective tissue In addition to forming the structural basis for the amorphous intercellular substance they also determine the distribution of water and electrolytes in the connective tissue They are thus responsible for the maintenance

Table I Alterations in content of hexosamine and hydroxyproline and in hexosamine to hydroxyproline ratio in skin from 14 untreated patients

	Hexosamine (μg/mg)[1]	Hydroxyproline (μg/mg)	Ratio
Initial values	3.94 ± 0.15[2]	95.3 ± 4.8	0.042 ± 0.001
Values after one week	4.06 ± 0.24	93.1 ± 4.4	0.044 ± 0.002
Values after two weeks	3.95 ± 0.23	92.2 ± 3.7	0.043 ± 0.002
Changes after one week	0.12 ± 0.19	−2.2 ± 2.9	0.002 ± 0.002
Changes from one to two weeks	−0.11 ± 0.22	−1.0 ± 2.1	−0.001 ± 0.003
Changes after two weeks	0.01 ± 0.20	−3.2 ± 2.2	0.001 ± 0.002

μg/mg dried, defatted tissue
Mean ± standard deviation of mean
For further details see (13)

of a constant environment for the cells. Furthermore, because of their electric charges and the intermolecular forces they are of importance in the organization of the cells and other structural components of the connective tissue, for example the collagen fibres. Finally, they are of significance in the course of a number of enzymatic processes. Because of the important functions of the mucopolysaccharides great efforts have been devoted to the biochemical studies of these compounds and to the elucidation of the regulative factors which control their metabolism.

It has become apparent that high concentrations of glucocorticoids inhibit the synthesis of mucopolysaccharides, including the acid mucopolysaccharides, decreasing the amount of these substances in the tissue. The inhibition occurs at the cellular level, the acid mucopolysaccharides being produced in the connective tissue cells. This effect has been illustrated in some studies (13, 14) which we have carried out on human skin and vein biopsies before and after 2 and 3 weeks of prednisone therapy. The total amount of mucopolysaccharides was assessed by a hexosamine analysis (table I) and the amount of collagen by a determination of the imino acid hydroxyproline which is specific to collagen. It may be seen that 2 weeks of prednisone therapy cause a fall in the hexosamine content and in the hexosamine to hydroxyproline ratio as compared with the findings in untreated control patients (table II). Similar changes (tables III and IV) were found in the vein biopsies in which there was a fall in the hexosamine to hydroxyproline ratio after 3 weeks of treatment, indicating a reduction in the amount of mucopolysaccharides in proportion to the amount of collagen. These investigations suggest that there is also a general effect on the connective tissue in man following therapeutic doses of glucocorticoids.

In evaluating the apparently small quantitative changes in the mucopolysaccharides, the profound influence of the polyelectrolytes upon their environment must be taken into consideration. This implies that the small quantitative changes in the mucopolysaccharides may induce extensive alterations in the connective tissue. Because of this phenomenon the changes in the mucopolysaccharides in the con

Table II Alterations in content of hexosamine and hydroxyproline and in hexosamine to hydroxyproline ratio in skin from 13 patients treated for two weeks with prednisone

	Hexosamine (μg/mg)	Hydroxyproline (μg/mg)	Ratio
Initial values	3.93 ± 0.13[3]	99.0 ± 4.9	0.041 ± 0.002
Values after one week	3.85 ± 0.13	101.8 ± 5.6	0.039 ± 0.002
Values after two weeks	3.57 ± 0.10	102.4 ± 5.1	0.036 ± 0.002
Changes after one week	0.08 ± 0.16	2.9 ± 2.2	−0.002 ± 0.002
Changes from one to two weeks	0.29 ± 0.16	0.5 ± 3.9	−0.003 ± 0.002
Changes after two weeks	0.36 ± 0.16	3.4 ± 3.7	−0.005 ± 0.001

μg/mg dried, defatted tissue
[2] Mean ± standard deviation of mean
[3] The reduction is statistically significant with p less than 0.05
The reduction is statistically significant with p less than 0.001

Table III Content of hexosamine and hydroxyproline and hexosamine to hydroxyproline ratio in human vein biopsies from 10 untreated patients

	Hexosamine (μg/mg)	Hydroxyproline (μg/mg)	Ratio
Initial values	5 78 ± 0 15	61 2 ± 2 6	0 097 ± 0 006
Values after 3 weeks	6 09 ± 0 19	66 3 ± 2 1	0 094 ± 0 006
Changes after 3 weeks	0 31 ± 0.28	5 12 ± 3 95	−0 003 ± 0 010

Calculated from the individual differences between first and second biopsies
Micrograms per milligram of dried defatted tissue
Mean ± standard deviation of mean
For further details see (14)

nective tissue induced by the glucocorticoids are considered as important causes of a number of the clinical effects observed during treatment with high doses of glucocorticoids This is true of such effects as osteoporosis vascular fragility gastric ulcer changes in the water and electrolyte balance of the body, inhibition of wound healing and to some extent the anti inflammatory effect

The glucocorticoids exert a similar inhibitory effect on the synthesis of collagen (11 12 17) The inhibition is primarily mediated *via* the fibroblasts in which the primitive collagen molecule is formed The effect on the collagen synthesis is presumably merely one of the manifestations of the anti anabolic effect of the glucocorticoids on the proteins While young soluble collagen is greatly influenced by the glucocorticoids the same is not true of the mature and insoluble collagen although some workers claim that the glucocorticoids may increase the degradation of collagen (10) The effect of glucocorticoids on collagen is therefore particularily prominent in active synthesis such as that in young granulation tissue The reduced synthesis of collagen is reflected in the inhibition of wound healing and the decreased tensile strength of the wound It is thus apparent that the general inhibitory effect of the glucocorticoids primarily exerted on the mesenchymal cells and secondarily on the extracellular tissue is particularly marked when the connective tissue is in an active proliferative phase as in wound healing and inflammation This phenomenon forms the basis of the clinical effect of the glucocorticoids in those disorders in which an important role is played by non specific reparative and inflammatory processes in the connective tissue for example the so-called connective tissue disorders

The question of the anti inflammatory effect of the glucocorticoids (1 4 6 7 15 16) is however more comprehensive In this connection it is natural to deal with the inhibitory effect of the glucocorticoids on the immunological reactions in the connective tissue (5 8 9) However the non specific inhibitory effect on the inflammation in connective tissue is probably of the greatest clinical importance regardless of whether the inflammatory reaction is induced by toxic or ischaemic tissue damage or an antigen antibody reaction While it is possible to give a purely descriptive account of the inhibitory effect of the glucocorticoids on the various phases of the inflammatory reaction our knowledge of the basic mechanisms involved is very deficient An interesting theory suggests that one of the earliest

Table IV Content of hexosamine and hydroxyproline and hexosamine to hydroxyproline ratio in human vein biopsies from 18 patients treated for 3 weeks with prednisolone

	Hexosamine (μg/mg)	Hydroxyproline (μg/mg)	Ratio
Initial values	5 62 ± 0 14	65 9 ± 2 6	0 088 ± 0 004
Values after 3 weeks	5 34 ± 0 15	68 2 ± 2 1	0 080 ± 0 004
Changes after 3 weeks	−0 28 ± 0 16	2 33 ± 2 3	−0 008 ± 0 003[4]

Calculated from the individual differences between first and second biopsies
Micrograms per milligram of dried defatted tissue
Mean ± standard deviation of mean
The reduction is statistically significant with p less than 0 05

effects of the glucocorticoids on inflammatory reactions is a *stabilization of the lysosome membrane* The lysosomes are subcellular enclaves containing hydrolytic enzyme systems which on their release from the cells are capable of inducing tissue damage and thereby inflammatory reactions However the glucocorticoids also affect the later stages of the inflammatory reactions including the vascular and cellular phases

The inflammatory oedema and the migration of leucocytes from the blood vessels are reduced *via* an inhibition of the capillary arteriole and venule dilatation The mechanism of this inhibitory action has not been elucidated Possibly the glucocorticoids exert a direct effect on the endothelial cells Furthermore the steroid induced sensitization of the smooth muscle cells of the blood vessels to the catecholamines may be of importance Where the treatment with glucocorticoids is of long duration the reduced production and release of chemical transmitters such as histamine and bradykinin in the inflammation may play a role

The migration of leucocytes from the vascular system is inhibited This is presumably partly due to the reduction in the permeability of the capillaries and partly to a direct effect on the polymorphonuclear leucocytes There is a reduction in their ability to adhere to the capillary endothelium and also in their pseudopodial activity Phagocytosis is inhibited as is their ability to digest already phagocytosed material The monocytes and extravascular macrophages are affected in a similar manner

The effect of the glucocorticoids on the lymphocytes is very radical There is a fall in the number of lymphocytes in both the blood and tissues as the result of increased destruction and reduced production Oedema and later involution of the lymphatic tissue occurs As is the case with the other cell types there seems to be a general inhibitory effect on the cell metabolism especially on the protein metabolism The plasma cells are also subjected to this influence Because of the effect on the protein synthesis of the lymphocytes and plasma cells the antibody production is reduced This is most obvious following the primary antigenic stimulus, and less so after the secondary stimulus On the other hand the glucocorticoids apparently have no effect on the binding of antigen to antibody on preformed antibodies or on the antigen itself Accordingly the effect of the glucocorticoids on the immunological reactions associated with humoral hypersensitivity is not very prominent and not observed until the reduced production *of antibodies or chemical transmitters has* become manifest By contrast the glucocorticoids induce an obvious inhibition of the cellular hypersensitivity which is associated with the lymphocytes However there is no doubt that with regard to the anti allergic action of the glucocorticoids the non specific anti inflammatory effect plays an important part

The effects of the glucocorticoids described here must be taken into account in the evaluation of their action on the various disorders The primary effect of the glucocorticoids will usually be an inhibition of the reaction of the body to some kind of damage Perhaps the resistance to the original as yet unknown aetiological factor will thereby also be reduced Glucocorticoid therapy can be considered to be an adequate form of treatment when the disease is due primarily to immunological reactions followed by secondary inflammatory processes If this is not the case then the treatment is purely symptomatic affecting first and foremost those manifestations of the disorders which are directly dependent on secondary immunological reactions and secondary reparative and inflammatory tissue reactions

DISCUSSION

Dr Lorenzen In connection with some of the problems which have been dealt with here it would seem reasonable to discuss the question of the anti anabolic and catabolic actions of the glucocorticoids Should the action on the protein compounds and thereby also on for example collagen be considered merely as the result of an anti anabolic effect or have the glucocorticoids also any catabolic effect?

Dr Binder I believe that the anti anabolic effect is the most prominent We know that the inhibitory influence on wound healing has been demonstrated in animal experimental studies Is there a corresponding inhibition of wound healing in human clinical practice?

Dr Lorenzen The practical clinical importance of the inhibitory effect of the glucocorticoids on wound healing has probably been exaggerated The doses which have been used in the animal experiments on wound healing have generally been considerably higher than those which are used in clinical practice in man

REFERENCES

1 Allison F Anti inflammatory agents In The inflammatory process B W Zweifach L Grant & R T McCluskey eds p 559–76 Academic Press New York & London 1965

2 Asboe Hansen G Mechanism of steroid inhibition of connective tissue growth In Inflammation and diseases of connective tissue L C Mills & J H Moyer eds p 460–64 Saunders Philadelphia and London 1961

3 Asboe Hansen G Connective tissue Ann Rev Physiol 25 41–60 1965

4 Bollet A J Metabolic changes during inflammation and the effect of anti inflammatory agents In Inflammation and diseases of connective tissue L C Mills & J H Moyer eds p 465–72 Saunders Philadelphia and London 1961

5 Cope C L Steroids inflammation and immunity In Adrenal steroids and disease C L Cope ed p 255–81 Pitman London 1965

6 Dougherty T F Role of steroids in regulation of inflammation In Inflammation and diseases of connective tissue L C Mills & J H Moyer eds p 449–60 Saunders Philadelphia and London 1961

7 Favour C B The cellular response to inflammation and its modification by glucocorticoids Ibid p 486–501

8 Fischel E E Inhibition of antibody production by cortisone Ibid p 472–77

9 Fischel E E Effect of adrenocorticosteroids on various types of antigen antibody reactions Ibid p 477–82

10 Houck F C & Patel Y M Proposed mode of action of corticosteroids on the connective tissue Nature 206 158–160 1965

11 Kivirikko K I Hydroxyproline-containing fractions in normal and cortisone treated chick embryos Acta physiol scand 60 suppl 219 1963

12 Laitinen O The metabolism of collagen and its hormonal control in the rat Acta endocr (Kbh) 56 suppl 120 1967

13 Lorenzen I & Zachariae H Effect of glucocorticoids upon the mucopolysaccharide content of human skin Acta med scand 179 337–41 1966

14 Lorenzen I & Hansen L K Effect of glucocorticoids on human vascular connective tissue Vascular Dis 4 335–41 1967

15 Rebuck J W Smith R W & Mellinger R C Modification of the leucocyte response in man by ACTH In Inflammation and diseases of connective tissue L C Mills & J H Moyer eds p 502–06 Saunders Philadelphia and London 1961

16 Spain D M Steroid alterations in the histopathology of chemically induced inflammation Ibid p 514–17

17 Wegelius O & Kock B The basic mechanism in the effect of cortisone on the connective tissue In Hormones and connective tissue G Asboe Hansen ed p 70–81 Munksgaard Copenhagen 1966

Acta med scand Suppl 500 pp 23-28 1969

GLUCOCORTICOIDS IN RHEUMATIC FEVER AND RHEUMATOID ARTHRITIS

by
Eigill Hvidberg

When the glucocorticoids were introduced as thera peutic agents at the end of the 1940s by Hench and his colleagues it was because of their pronounced anti inflammatory properties After twenty years of experience rheumatic fever and rheumatoid arthritis can therefore be considered as the classical in dications for glucocorticoid therapy After the first years of fluctuation between triumph and disappoint ment the treatment of these disorders with glucocor ticoids has gradually reached its present state From a clinical point of view there have been no great advances during the past five or six years In con trast an almost explosive development has taken place in a number of the fields of basic research into these hormones This applies mainly to pharma codynamics and modes of action but as yet these developments have had no important influence on the practical employment of the glucocorticoids as anti inflammatory and anti immune drugs This is particularly true of rheumatic fever and rheumatoid arthritis which are described in the following in relation to steroid therapy

The incidence and mortality rate of *rheumatic fever* has shown a very pronounced fall within the last generation This development has correctly been called a social triumph more than anything else as it cannot be attributed to penicillin and certainly not to steroid therapy The main problem seems to be whether an acute case should be treated with salicylates or with glucocorticoids Treatment is aimed at a reduction in the severity and possibly also the duration of the signs and symptoms of the acute attack It is also aimed at the prevention of sequelae first and foremost damage to the valves of the heart The effect of both the salicylates and the glucocorticoids is symptomatic and not causal They do not reduce the duration of the acute attack although both forms of therapy are capable of pro ducing a significant decrease in the severity of the symptoms (25) It has now been clearly demonstrated that there is no difference between these treatments with respect to the incidence of sequelae in the form of rheumatic valvular disease

In a recent extensive multi-clinic study from Eng land and U S A a total of about 500 children were divided into three equal groups treated with sali cylates ACTH and cortisone respectively (28) Follow up studies after ten years revealed no differ ence between the groups with regard to permanent cardiac damage It was however claimed that the steroid dosage was too small and the period of administration too short (a small dose for only about six weeks) but the same results have been reported by other groups Thus in a smaller study with 57 patients two comparable groups received aspirin and prednisone respectively in high doses for twelve weeks (7) The result was exactly the same In the long run steroid therapy has therefore no advan tages and against this must be set the risk of serious side-effects In rheumatic fever the indication for the use of steroids can therefore only be the extremely serious acute case with severe exudative carditis which cannot be controlled by salicylates The cited results thus support recognised clinical experience

If it is nonetheless decided to initiate glucocor ticoid therapy in an acute case of rheumatic fever the duration of treatment should be as short as possible although it must still cover the whole course of the attack About 80 % of attacks will end within the first six weeks and practically all of the remainder will have terminated within the following four weeks A suitable maximum treatment would thus seem to have a duration of eight to ten weeks with gradual reduction over the following two to four weeks The dosage should be just sufficient to maintain the patient in a symptom free state (25)

The problems in *rheumatoid arthritis* are some

what different because of its chronic nature In this disorder the action of glucocorticoid therapy is also purely symptomatic and the rationale for the use of these drugs may therefore be questioned In fact we may even add gravely to the troubles of the patient In spite of this knowledge one is sometimes forced to turn to the glucocorticoids in rheumatoid arthritis and the steroids certainly exert the most dramatic symptomatic effect The limitation on their use is set by their highly undesirable side-effects and it is necessary severely to restrict their administration This is now generally acknowledged It is principally the long term therapy which causes the problems There is however very little reliable information about the justification of well organized steroid therapy of long duration and with small doses In this connection it is very important to take into consideration the qualitative aspects If such treatment can give the patient a better life less pain or perhaps a partial working capacity of some duration then it may be justified despite the obvious increase in the risks A study from 1961 (2) revealed precisely this very important aspect and the above mentioned types of improvement were noted in many of the patients even in some of those with the most severe disease However at the same time there was clear evidence that the disease progressed independently of the treatment and that there were marked side-effects Somewhat in contrast to these observations was the report in another of the early studies (17) that after two years no difference could be demonstrated between the results of intensive salicylate therapy and cortisone in moderate doses in patients with early rheumatoid arthritis The side effects were not alarming. These and other investigations exemplify and confirm our present point of view This is that either the corticosteroids exhibit no obvious advantages or the improvement is only to be gained at a great risk A more comprehensive view of this problem was put forward by Rothermich (24) in a very thorough study from 1964 He reported fewer complications with long term steroid therapy than with gold or phenylbutazone in a controlled programme of long term therapy In general the principles behind his endeavours are identical with good medical practice today but in this study they are supported by an admirable statistical material and a sober discussion

Although the lines of treatment employed are generally accepted they will be stated in brief 1) Long term therapy with glucocorticoids should be instituted only if an intensive steroid free programme has proved inadequate Accordingly the selection of the patients must be very careful 2) The glucocorticoids should be used only as a part of a consistent comprehensive programme under close medical supervision 3) As a rule prednisone should be the drug of choice although patients may occasionally find other glucocorticoids preferable 4) It is of utmost importance to use the lowest possible dosage 5) It might be advantageous occasionally to supplement the therapy with a limited number of intra articular injections of steroid 6) A concurrent anti-catabolic regime may be maintained although the usefulness of this is debatable

Despite this somewhat more optimistic evaluation of steroid therapy in rheumatoid arthritis it implies no encouragement for an uncritical use On the contrary it is in fact this which is the greatest danger

Concomitant administration of other drugs is sometimes tried in order to counteract some of the undesirable side-effects of long term therapy with glucocorticoids A number of reports advocate the simultaneous treatment with anabolic steroids (1 6 20 26) but the advantages of this therapy are debatable and it has not won general support The effect on the parameter most frequently measured the nitrogen metabolism seems to be transient (1) The effect of vitamin D on the negative calcium balance also seems to be temporary (22) One should be aware of the possibly dangerous impact of long term therapy with these substances on the kidneys and liver which are from a clinical point of view generally spared by the corticosteroids More evidence should be available before such agents are included in a routine programme In connection with the mention of the anabolic steroids it should be stated that methandrostenolone (methandienon (NFN) Dianabol ®) when given together with the anti inflammatory oxyphenbutazone seems to increase the concentration of the latter in the plasma If methandrostenolone is replaced by prednisone this effect is not seen (8) Furthermore it has been reported that the free form of the endogenous glucocorticoid in the plasma is increased during treatment with non steroid anti rheumatic drugs This mechanism might explain their anti inflammatory effect (3) but other investigations tend to suggest that this is an oversimplification (18) However these studies undoubtedly demonstrate different forms of drug interaction between the non steroid anti rheumatic drugs and the glucocorticoids and other steroids

although it is far too soon for this to have any therapeutic consequences On the other hand there is no doubt that aspirin in sufficient doses acts synergistically with the glucocorticoids in the thera peutic action in rheumatoid arthritis (12 15) By making use of this fact the steroid dosage and thus the risks involved can be reduced However simul taneous administration of corticoids and for example phenylbutazone or indomethacin may increase the incidence of complications particularly those from the stomach (24) although the results are conflicting

It has already been mentioned elsewhere in this symposium that modifications of the hydrocortisone molecule do not seem to have reduced the incidence of side-effects In fact the opposite is more likely to be true The considerable risk may be appreci ated from the finding of a total of 11 deaths out of a material of 50 patients treated with long term glucocorticoid therapy at the University Hospital Copenhagen (4 5) Although by no means proved it would seem reasonable to assume that a relative adrenal insufficiency may have contributed to the fatal outcome in some of these cases For such reasons experiments are being carried out in many places with the aim of changing the usual method by which glucocorticoid therapy is administered i e in three or four divided daily doses The hope is to find a means of avoiding suppression of the endo genous production of glucocorticoids Various inter mittent regimens have been tested In some the entire daily dose is given in the morning in the hope of exerting as little suppressive effect as possible by adjusting the exogenous administration to the natural diurnal variation in corticoid secretion (9 10 13 23) The results have been conflicting Other investigators have given one dose every other day the amount of the dose corresponding to the total which would have been given in small portions over the same period (13) The clinical effect was claimed to be unchanged with less adrenal suppression In these studies the patients suffered mainly from asthma and other non rheumatic diseases and it is therefore impossible to draw any conclusions about patients with rheumatoid arthritis On the contrary it would seem to be mainly patients with asthma and other allergic disorders for whom this dose schedule is suitable Most likely there are relatively few cases of rheumatoid arthritis which can be treated in this manner (11 30) At all events it would seem that patients in whom the disease is very active and those who require a daily dosage of more than 15–20 mg prednisone gain nothing by this form of therapy This is probably the most important factor in the case

A third form of intermittent therapy is to give a few days of treatment followed by a few days pause e g 7 + 4 days or 3 + 4 days (11) The number of patients involved in these studies has been limited and there have been no controls The authors stress however that the exacerbations on the dose free days during the first period of treat ment disappeared as the regimen was continued Only moderate suppression of the pituitary adrenal function was reported An interesting approach has been the use of intramuscular injections of glucocor ticords at intervals of weeks (14 29 30) The results were to some extent encouraging as about one quarter of the patients studied stated that the effect was more satisfactory than that of other forms of steroid therapy Both gastro intestinal and other side-effects seemed to be less severe However knowing how difficult it is to evaluate and compare such findings in different forms of anti rheumatic therapy (21) it must be justified to remain uncon vinced until these claims have been repeatedly con firmed in larger materials The pituitary adrenal sup pression was also said to be reduced This is not easy to understand as the action was due to a depot effect and it was of importance that an interval of about three weeks was maintained Prednisolone derivates of the type with several substitutions e g fluor methyl prednisolone were found to be most suitable for administration in this way A possible explanation may be offered by the fact that such derivatives are metabolised at a much slower rate as previously emphasized in this symposium To summarize it may be stated that if a moderately active rheumatoid arthritis seems to require gluco corticoid therapy an attempt may be made to give larger doses at longer intervals or to introduce pauses in the treatment However only a small number of patients with rheumatoid arthritis seem to benefit from such regimens As restitution of the adrenal function to its normal level is not to be expected on the change from the usual to intermit tent dosage the latter should be instituted only in patients who have not been receiving glucocorticoid therapy during the immediately preceding period The latest experiences of intermittent therapy from the Mayo Clinic also make this clear and are other wise not particularly encouraging (27)

In studies such as those described above it is of the utmost importance to assess the integrity of the hypothalmic pituitary adrenal axis This is particularly true during long term treatment of patients with rheumatoid arthritis and other rheumatic disorders with glucocorticoids Investigations into such problems are discussed in detail by Dr Binder elsewhere in this symposium It would seem that the hypothalamic pituitary part of this axis is the more sensitive to suppression caused by the exogenous administration of glucocorticoids and that it is also this part which is involved first The metopirone test and insulin induced hypoglycaemia are reliable sensitive tests which expose functional defects in this endocrine axis In particular the latter is easily performed (16) Such investigations should therefore constitute a natural part of any department s supervision of patients receiving long term therapy with glucocorticosteroids (19)

DISCUSSION

Dr Loren en I am surprised that the glucocorticoids have not played any part in the reduction of the lethality of rheumatic fever I would have thought that the verified beneficial effect of the glucocorticoids on severe rheumatic carditis where the time factor is so important in therapy must have been manifest in a reduction in the mortality rate

Dr Andresen It is interesting that salicylic acid acts synergistically with the steroids Is it not possible that the effect of salicylic acid alone in rheumatic fever is also due to the fact that salicylic acid stimulates the hypophysis and adrenal cortex? At all events there is an increase in the excretion of 17 ketogenic steroids in the urine

Dr Hvidberg In answer to the second question I can say that it is quite correct that salicylic acid has this effect but it has never been possible to demonstrate that it is of any importance as it very rapidly diminishes Furthermore in many experimental models the anti inflammatory effect of cortisone and the salicylates are different, and phase-displaced With regard to rheumatic carditis it is expressly stated in a number of reviews of this subject that the steroids have played no part in the change in lethality I have no personal experience of this

Dr Loren en Has there been no controlled investigation?

Dr Hvidberg No not insofar as I am aware

Dr Loren en Does the administration of the glucocorticoids instead of salicylates make any difference to the incidence of cardiac late sequelae in the form of valvular disease?

Dr Hvidberg No

Dr Loren en But with regard to acute rheumatic carditis, is there a lack of information?

Dr Olesen We have in fact little experience of rheumatic carditis in Denmark but at all symposia in which the subject arises the advice is given to use steroids in the very severe acute case It is possible to see a miraculous improvement in a very short time

On the other hand I know of no statistics which give any indisputable evidence such as that which Lorenzen is seeking But I also think that this is because the lethality in acute carditis has fallen very sharply and it would therefore be necessary to collect very large materials in order to demonstrate statistically that there is a difference

Dr Loren en It must be concluded from this that in this part of the world there are very seldom indications for the use of glucocorticoids in rheumatic fever

Dr Olesen Yes I would think so although I would at the same time recommend that they should be used if it is really considered that the case is life-endangering

Dr Loren en Hvidberg gave an account of two large materials of patients with rheumatoid arthritis The conclusion of one of the investigations was that it was not possible to achieve anything with the steroids which could not equally well be achieved with other forms of treatment and that the price of glucocorticoid therapy was a number of serious side effects The conclusion of the second study was more on the lines that the glucocorticoids should nonetheless be used in certain patients Is it possible to give a sharp delimitation of the presumptive indications for treatment in these patients?

Dr Hvidberg There are very few such indications Many workers have stated that in the end about 5–10 / of all patients with rheumatoid arthritis receive glucocorticoids I do not believe that this is a true picture There may well be indications for steroid therapy when all other possible forms of treatment have been tried and where the requirement of treatment is imperative The greatest risk in this however lies in the stretching of the range of indications and the liberalization of steroid therapy as have often occurred

Dr Horning One of the problems which may be encountered is that very often one takes over a patient with rheumatoid arthritis who is receiving or has received glucocorticoid therapy for a number of years I have gained the impression that it is extremely difficult to wean these patients from the treatment once it has been instituted I would like to ask Hvidberg about his experience with regard to the withdrawal of treatment which has already been started And in addition whether the fact that the withdrawal of treatment is so difficult suggests an indisputable clinical effect which as yet cannot be demonstrated objectively?

Dr Hvidberg I can reply positively to the last part of the question I understand from the question that you would like an evaluation of to what extent it is at all justifiable to withdraw treatment very slowly from these patients

It is a general experience that it is extremely difficult to

reduce long term steroid therapy even if withdrawal is extended over six months or even several years Some of the patients gradually deteriorate and are therefore compelled to continue treatment

Dr Loren en The other explanation of the difficulties in reducing a long term steroid therapy is surely that this treat ment has conferred a new disorder on the patient and that this *per se* necessitates supplements of glucocorticoids

Dr Kalbak We have been particularly interested in patients receiving long term treatment In this connection I would like to ask how long term steroid therapy should be defined In our experience whenever one institutes steroid therapy in a patient with rheumatoid arthritis one starts on a very long term treatment

There are great problems associated with the reduction and withdrawal of treatment but this is possible Generally we carry it out over the course of 3-6 months but the patients do not return to normal again until at least one year after the withdrawal has been completed

Dr Hvidberg Long term therapy is usually defined as a treatment lasting for more than 3 months

Dr Mosbech Hvidberg mentioned in his paper that in patients with rheumatoid arthritis who are receiving steroid therapy this can be supplemented with intra articular injec tions of cortisone and cortisone preparations It has pre viously been considered that this was associated with a con siderable risk of infection is this still true?

Dr Hvidberg I am not a strong advocate of the use of intra articular injections of glucocorticoids but it has been demonstrated at a large centre in U S A where they used intra articular injections into the same joint at suitably long intervals (months) that infections are seldom observed

Dr Kalbak For how long should other methods of treat ment be used before one turns to steroid therapy?

Dr Hvidberg I am no more able than anyone else to give any definite rules The majority of workers prefer to treat rheumatoid arthritis with salicylates and later possibly with a series of gold injections Some use indomethacin or phenylbutazone for relatively short periods Rothemich s report which I quoted took a somewhat pessimistic view of gold therapy

Dr Loren en We must conclude that there is no ideal drug available for the treatment of rheumatoid arthritis, and that we know that the glucocorticoids cannot fulfil the require ments of such a drug. There are very few indications for the use of glucocorticoids in long term therapy in rheumatoid arthritis Treatment must be limited to the very severely invalided patients with requirements for treatment and in whom all other forms of therapy have proved to be in effective

REFERENCES

1 Aschkenasy A Effect of protein deficiency on the endocrine system In Protein metabolism an internatio nal symposium Springer Verlag, p 268 272, 1962

2 Berntsen Carl A & Freyburg Richard H Rheumatoid patients after five or more years of corticosteroid A comparative analysis of 183 cases Ann intern Med 54 938–953 1961

3 Brodie B B Displacement of one drug by another from carrier or receptor sites Proc Roy Soc Med 58 946–955 1965

4 Boye Nielsen J Drivsholm Aa Fischer F & Broch ner Mortensen K Long term treatment with corti costeroids in rheumatoid arthritis Acta med Scand 173 177–183 1963

5 Brochner Mortensen K Nerup J & Boye Nielsen J (As ref No 4 16 years follow up) 1967 to be pub lished

6 Clark, G & Mills D Corticosteroid therapy of rheu matoid arthritis supplemented with methandrostenolone Arthr and Rheum 5 156–162 1962

7 Combined Rheumatic Fever Study Group 1960 A com parison of the effect of prednisone and acetylsalicylic acid on the incidence of residual heart damage New Engl J Med 262 895–902 1960

8 Dayton P G Hvidberg E F Read J M & Wilson C H Interaction of phenylbutazone oxyphenbutazone and methandrostenolone in man Fed Proc 27 531 1968

9 Demos C H Krasner F & Groel J T Modified (once a day) corticosteroid dosage regime Clin Pharma col Ther 5 721–727 1964

10 Dubois E L & Adler D C Single daily-dose oral administration in rheumatic disorders An analysis of its advantages efficacy and side effects Curr Ther Res 5 43–56 1963

11 Fisher D A Panos T D & Melby J C Intermittent corticosteroid therapy of juvenile rheumatoid arthritis Arthr and Rheum 7 413–424 1964

12 Gum Oren B Controlled study of two preparations of paramethasone propoxyphene and aspirin and propo xyphene aspirin in the treatment of arthritis Amer J med Sci 251 328–332, 1966

13 Harter T G Reddy W J & Thorn G W Studies on an intermittent corticosteroid dosage regimen New Engl Med 269 591–596 1963

14 Hartfall S J Walker W C & Writht, V Intra muscular prednisolone trimethyl acetate therapy Ex perience in its use and complications Acta rheum scand 8 260–264 1962

15 Hercher J Pinals R S Ullian R Slone D & Muench H Dexamethasone and aspirin-dexametha sone in the treatment of chronic rheumatoid arthritis A controlled trial Lancet II 1203–1205 1965

16 Jasani Mukundrai K Boyle James At Greig, Wil liam R Dalakos Theodore G Browning, Margaret C K Thompson Anne and Buchanan Watson W Corticosteroid induced suppression of the hypothalamo pituitary adrenal axis Observations of patients given oral corticosteroids for rheumatoid arthritis I Quarterly Journal of Medicine New Series XXXVI 261–276 1967

17 The joint committee of the Medical Research Council and Nuffield Foundation on clinical trials of cortisone ACTH and other therapeutic measures in chronic rheumatoid diseases A comparison of cortisone and aspirin in the treatment of early cases of rheumatoid arthritis Brit med J 2 695–700 1955
18 Hvidberg E. Schou J Jansen J Aas & Clausen J E Tissue and plasma cortisol in man under various conditions *Acta med scand* 184 215–223 1968
19 Jørgensen Asger Whitta Hypofyse binyrebarkfunktion under langtidsbehandling med kortikosteroider Ugeskr Læg 127 44–47 1965
20 Kowalenski K Effect of steroids on bone formation in protein metabilis An International Symposium Springer Verlag p 238 262 1962
21 Leading Article Testing drugs for rheumatoid arthritis Brit med J 4 251–252 1967
22 Manson Gordon and Johnston Joseph A Studies in rheumatic fever IV Attempts to offset calcium losses incidental to steroid therapy Amer J dis Child 109 483–488 1965
23 Matthews H A Preliminary report on single daily dose corticosteroid therapy Med Tms (Lond) 90 934–939 1962
24 Rothermich Norman Corticosteroid therapy in rheumatic arthritis Postgrad Med 36 117–128 1964
25 Russell V Lucas Acute rheumatic fever A New Look The Journal Lancet 87 230–235 1967
26 Querido A Protein metabolism In Protein metabolism *an international symposium Springer Verlag p* 1–7 1962
27 Sones D A & Slocumb C H Steroid therapy in rheumatoid arthritis Med Clin Amer 52 739–745 1968
28 United Kingdom and United States Report The natural history of rheumatic fever and rheumatic heart disease Ten year report of a cooperative clinical trial of ACTH cortisone and aspirin Circulation 32 457–476 1965
29 West H F Long term intramuscular prednisolone therapy Ann rheum Dis 21 191–193 1962
30 Zuckner Jack and Ramsey Robert H Modified *method of steroid administration in arthritis Missouri* Med 64 29–33 1967

Acta med scand Suppl 500 pp 29-33 1969

GLUCOCORTICOIDS IN THE CONNECTIVE TISSUE DISEASES

by
Ib Lorenzen

The first demonstration of the dramatic clinical effect of pharmacological doses of the glucocorticoids al most 20 years ago was in the connective tissue diseases (12 13) Today these diseases still represent some of the commonest and most important indications for this treatment

The disorders of connective tissue in which gluco corticoid therapy may be indicated are the so-called collagen diseases This is a poorly defined group of diseases of unknown aetiology However the dis orders have two common features which are of particular importance in the evaluation of the effect of the glucocorticoids Firstly in all there are changes in the connective tissue which are to a great extent non specific including degenerative changes and necrosis and secondary to these inflammatory and reparative processes Secondly immunological reactions would seem to play a pathogenic role which is prominent in several of the disorders The frequently dramatic symptomatic effect of the glucor corticoids in these diseases is readily understandable on the basis of the ability of these hormones to inhibit inflammatory and immunological reactions On the other hand in consideration of the unknown aetiology of the diseases it is most probable that their action is merely symptomatic

There are great difficulties involved in the evaluation of the effect of the glucocorticoids on the course and prognosis of these disorders such that it is in fact almost impossible to make any reliable evaluation

Despite the wide spread use of the corticosteroids for almost 20 years there is still a lack of satisfactory control studies This is mainly due to the fact that the immediate effect on the more serious manifestations of these diseases is usually so dramatic that it has been considered impossible from an ethical point of view to withhold this therapy from the patients

Moreover the clinical picture in several of the connective tissue diseases has changed in character during the past 20 years This makes a comparison of the course of the disorders before and after the introduction of the glucocorticoids difficult There are several causes of the change in the clinical picture The number of diagnostic aids has increased as illustrated by for example the LE factor Furthermore physicians have become increasingly aware of the existence of this group of disorders These two factors have undoubtedly led to an increase in the number of recognized connective tissue diseases with less severe manifestations

Another difficulty in evaluation arises because of the marked tendency of the connective tissue diseases to spontaneous remissions which may occasionally be of long duration

Finally other forms of treatment of these diseases were introduced at about the same time as steroid therapy Of greatest importance is undoubtedly antibiotic therapy which has greatly intensified since 1940 On the evidence it would seem to have led to a marked improvement in the prognosis of several of the connective tissue diseases in which secondary bacterial infection is common and sometimes determinant in the fatal outcome

Systemic lupus erythematosus (SLE) is the most thoroughly studied of the connective tissue diseases and the results and problems connected with gluco corticoid therapy in this disease can on the whole be applied to other connective tissue diseases The value of the corticosteroid treatment of SLE will therefore be considered in more detail while the results of the treatment of the other diseases will be discussed only in brief

SYSTEMIC LUPUS ERYTHEMATOSUS

The treatment of SLE with glucocorticoids has been evaluated in a number of large materials particularly from America (table I) However none of these

Table I Studies on the effects of glucocorticoids in SLE*

Study	no patients	no receiving steroid therapy
Klemperer et al 1941(18)	20	0
Harvey et al 1954(11)	138	62
Dubois 1956(4)	163	132
Soffer 1961(29)	90	90
Dubois & Tuffanelli 1964(5) 1966(7)	520	409

* The studies also include reviews of a number of other similar investigations

materials include any real control groups In general patients whose disease began before the introduction of the glucocorticoids into therapy have been used as controls Furthermore the authors concerned have frequently not examined the patients personally The value of such control materials none of which is very large is limited One of the largest and best studied materials has been published by Dubois (4 5 7) His findings are in general in accordance with those reported by others and the discussion below is therefore based upon his results

The only indications for treatment with glucocorticoids have been clinical evidence of disease activity combined with a lack of effect of other treatment The preparations used have generally been the synthetic glucocorticoids although cortisone was used during the early years The dosage and the duration of treatment have depended only on the activity of the disease

It would seem natural to evaluate the effect of the *glucocorticoids on four parameters 1 the clinical* symptoms 2 the duration of illness 3 the causes of death 4 the histo-pathology of SLE

With regard to the clinical symptoms in his first treated series Dubois (4 7) obtained at least temporary effect in 90 % of his patients whilst 10 % proved resistent despite the administration of large doses of steroids for up to 6 weeks In half of the patients who responded the treatment had to be permanent while in the other half it proved possible to withdraw the treatment after an average of *12 5 months* However it must be taken into consideration that there is a tendency to spontaneous remission in SLE in about the same proportion of patients (4 6) Relapses were also observed in half of the patients in whom it was possible to withdraw steroid therapy after the first course of treatment. The symptomatic effect was found to be satisfactory in the majority of the manifestations of SLE, but was poor in advanced lupus nephritis It is of interest that neither the LE factor nor the ESR was a good indicator of the activity of the disease

The duration of the illness has been evaluated partly from the survival time and partly from the duration of the illness before closing of the study in the various investigations (table II) It can be seen that before the introduction of the steroids the five year survival rate was about 20 % In patients treated with glucocorticoids Dubois observed a five year survival of 45 % in 1956 and of 55 % in 1963 Correspondingly the average duration of illness rose from 24 months to 45 and 95 months respectively Although it is probable that the steroids have contributed to the improved prognosis there are reasons to emphasize the other factors which were named in the introduction in particular the importance of the LE factor in diagnosis and the antibiotic therapy

It may be seen from table III that the causes of death in SLE have changed during the past 20 years Before the introduction of steroid therapy infectious diseases were the most important causes of death followed by cerebral vasculitis Later uraemia and cerebral vasculitis came to the fore This is presumably a result of the longer survival allowing the vascular complications to develop In Dubois opinion (7) the fall in the number of cases of fatal cerebral vasculitis in 1963 as compared with 1956 is due to the use of large doses of glucocorticoids in this manifestation of the disease

Table II Duration of illness in SLE before and after introduction of glucocorticoids into therapy

Study	Jessar et al 1953 103 pts (17)	Dubois, 1956 60 pts (4)	Dubois 1956 163 pts (4)	Dubois 1964 5_0 pts.(5 7)
5 year survival	20 /	20 /	40 /.	55 /
Duration of illness (mths)		24 (36 pts)	45	95
Relation to introdu tion of steroids	Before		After	

* Average duration illnes to death or completion of study

Table III Causes of death in SLE before and after introduction of glucocorticoids in therapy

Causes of death	Klemperer et al 1941 20 pts (18)	Dubois 1956 58 pts (4)	Dubois 1964 135 pts (5 8)
Uraemia	5*	33	34
Cerebral vasculitis	25	43	20
Infections	60	3	12
Other causes	10	21	34

* Percentage

Despite the longer survival times of the treated groups it has not been possible to demonstrate any difference in the histopathological findings in untreated and steroid treated patients who have died of SLE (26) This is in accordance with the purely suppressive action of the steroids In contrast Pollak & Pirani (22) found an improvement in the histological picture as assessed from repeated renal biopsies in 31 patients with lupus nephritis who were treated with large doses of prednisone

The published reports would thus suggest that the glucocorticoids have a convincing symptomatic effect on the majority of the manifestations of SLE On the other hand it is not proved that this treatment has any effect on the duration of the illness and the prognosis However on the basis of the effect on the more serious manifestations of the disease it is probable that the glucocorticoids are capable of inhibiting the development of vascular and visceral damage and in this way of improving the prognosis As the treatment is symptomatic the only guide to treatment is evidence of activity of the disease

POLYARTERITIS AND TEMPORAL ARTERITIS

The results of the treatment of polyarteritis are even more difficult to evaluate than those of SLE partly because the spontaneous course of polyarteritis is more variable and partly because the disease is rare There are no true controlled studies but in some reports the effect of the steroids has been compared with more or less suitable control groups investigated before the introduction of steroid therapy (9 23 24) On the whole the survival time would seem to be somewhat longer in patients treated with steroids than in those who did not receive this treatment (9) However in one material it was found that this difference was only apparent after one year of treatment whilst after three years of treatment the percentage survival was no greater in the group treated with steroids than in the untreated group (23 24)

The effect of glucocorticoids on the course of the disease is more convincing in temporal arteritis both in its pure form and in association with polymyalgia rheumatica in which it is reported in about 20–50% of cases (2 3 10 19) One of the most serious complications of temporal arteritis is impairment of vision which occurs in about 40% of untreated patients (1 14 31) Several reports of large materials (1 14) have demonstrated that an effective and long term steroid treatment can almost prevent this complication although the glucocorticoids would seem to have no influence on the duration of the illness

This observation suggests that it may also be possible to inhibit the development of serious vascular damage in SLE and polyarteritis despite the fact that the duration of the illnesses is unaffected

DERMATOMYOSITIS – POLYMYOSITIS

The effect of the glucocorticoids in dermatomyositis and polymyositis has been evaluated by means of retrospective analysis of a number of large materials (20 21 25) The steroids have no convincing effect on the duration of illness and presumably similarly no effect on the prognosis The immediate clinical effect to some extent depends on the clinical type of the illness (25) but it is often dramatic and improvement may be observed in up to half of the patients treated (25) Recurrence after the withdrawal of steroid therapy is common particularly within the first year (25) As a rule it is necessary to use large doses of glucocorticoids initially and treatment is often necessary for several years the length being determined solely by evidence of activity of the disorder

SARCOIDOSIS

Some workers include this disease among the con nective tissue diseases

In contrast to the disorders mentioned above sarcoidosis often has a good spontaneous prognosis (28) Treatment is indicated only when the vital organs (central nervous system eyes heart lungs) are involved and/or the disease shows evidence of progression In several large though uncontrolled series it has been found that the glucocorticoids have hardly any effect on either the duration of the illness or the prognosis (15 27 28 30) The chance of spontaneous remission is apparently not increased by treatment with the glucocorticoids in the early stages of the illness (15) but remission occurs more rapidly than the spontaneous remission (28) Where the illness has lasted for more than 3 years the chance of spontaneous remission is small In such cases the glucocorticoids may inhibit progression and occa sionally also improve the condition (15 27) It is interesting that a double blind investigation using prednisone and oxyphenylbutazone revealed that the oxyphenylbutazone exhibited the same beneficial ef fect as prednisone (16) This confirms the impression that in both cases the effect is due to a non specific anti inflammatory action which leads to suppres-sion of the disease but not to cure Because of the marked tendency to spontaneous remission steroid therapy should usually not be given until the patient has been observed for one year (28)

On the basis of the published studies it may be concluded that as a rule the glucocorticoids can in hibit the activity of the connective tissue diseases This is primarily due to the anti inflammatory effect of the steroids It is doubtful whether there is any effect on the duration of the illness or on the mor tality rate However it is probable that the extent of the serious vascular damage to the organs can be reduced and the prognosis thereby possibly im proved As the effect of the glucocorticoids is clearly symptomatic the only guide to therapy must be the activity of the disease and treatment must be limited to the least possible The indications for treatment must be balanced against the knowledge that long term treatment with glucocorticoids in pharmacolog ical doses can induce new disorders which may be lethal

DISCUSSION

Dr Rossel Isn t steroid therapy always indicated in poly myalgia rheumatica?

Dr Loren en In my opinion the indications for steroid therapy in polymyalgia rheumatica are entirely dependent upon whether or not the patient also has temporal arteritis This may be very difficult to ascertain In some materials *the incidence of temporal arteritis in polymyalgia rheumatica* is stated to be as high as 50–80 % whilst other workers have found a much lower incidence It is probably true that temporal arteritis may occur as a manifestation of poly myalgia rheumatica or that the two clinical pictures are aspects of the same basic disorder As the indication for steroid therapy in temporal arteritis is indisputable this must presumably indicate steroid therapy in the majority of cases of polymyalgia rheumatica However I would consider that biopsies from the temporal arteries should be taken in these patients If there is no evidence of temporal arteritis in these then I would consider it justifiable to refrain from giving steroid therapy

Dr Andresen I would like to enquire about the dosage of steroids which should be used in the treatment of temporal arteritis Insofar as I can understand it is important that the dosage of glucocorticoids is sufficient and that the treatment is of long duration

Dr Loren en It is general experience that one must be guided by the evidence of disease activity This is true of temporal arteritis and equally of the other connective tissue diseases

In temporal arteritis the majority of workers start with doses which for prednisone are in the region of 50 mg daily If the patient does not react rapidly to this dosage then it must be increased immediately It holds true of both tem poral arteritis and the cerebral vasculitis seen in SLE that the requirements for treatment are imperative and that therapy is urgent because it makes it possible to reduce the serious parenchymatous damage which results from active vasculitis The treatment should be continued until with drawal no longer leads to a recrudescence of the disease activity

Dr Videbæk Is it possible to give a more general outline of the indications for steroid therapy in the connective tissue diseases?

Dr Loren en When there are vascular manifestations from vital organs such as the central nervous system heart lungs *and kidneys or from the eyes then there is an indication for* the immediate start of treatment with glucocorticoids If on the other hand less vital organs are involved it would be reasonable to try some of the other forms of treatment which are available in these conditions for example the salicylates and antimalarials

With these latter drugs it is often possible to obtain the same symptomatic effect as with the glucocorticoids and there are fewer side effects

Dr Andersen For the sake of completeness one should per haps mention hypercalcaemia in Boeck s disease as an indi cation for steroid therapy

Dr Asboe Hansen I agree with what Lorenzen has said but it is possibly necessary to emphasize that it is useless to treat a disease process which is no longer active I mean for in stance a fibrotic burnt out scleroderma On the other hand it is certainly possible to treat exudative and acute inflam matory processes There is little doubt that in materials which have been collected for evaluation of the effect of

glucocorticoids a considerable number of burnt-out cases have been included This greatly reduces the value of such materials

Dr Nissen You mentioned the pulmonary changes in Boeck s disease as an indication for therapy How extensive do you consider such changes should be before steroid therapy is initiated and how long should treatment be continued?

Dr Loren en The question is difficult to answer and as far as I can see it has not yet been clarified However it is extremely important in this disease that the pronounced tendency to spontaneous remission and the relatively good prognosis are taken into consideration Most workers make use of steroid therapy when the pulmonary symptoms have shown *convincing evidence of progression for one year* By this means it is possible to prevent progression for a certain length of time and to achieve some improvement in the pulmonary function It is true that it would seem that if steroid therapy is initiated within one year of the onset of the disease it is possible to induce remission more rapidly than without this therapy but the incidence of spontaneous remission is not increased The duration of treatment must as in all other diseases of connective tissue depend solely on the activity of the disease

REFERENCES

1 Birkhead N C Wagener H P & Shick R M Treatment of temporal arteritis with adrenal corticosteroids J Amer med Ass 163 821-27 1957

2 Bruk M I Articular and vascular manifestations of *polymyalgia rheumatica Ann rheum Dis 26 103-16* 1967

3 Dixon A St J Beardwell C Kay A Wanka J & Wong Y T Polymyalgia rheumatica and temporal arteritis Ann rheum Dis 25 203-08 1966

4 Dubois E L Systemic lupus erythematosus recent advances in its diagnosis and treatment Ann intern Med 45 163-84 1956

5 Dubois E L & Tuffanelli D L Clinical manifestations of systemic lupus erythematosus Computer analysis of 520 cases J Amer med Ass 190 104-11 1964

6 Dubois E L The clinical picture of systemic lupus erythematosus In Lupus erythematosus E L Dubois ed pp 129-276 Mc Graw Hill New York 1966

7 Dubois E L Results of steroid therapy in systemic lupus erythematosus Ibid pp 388-402

8 Dubois E L Causes of death in systemic lupus erythematosus Ibid pp 403-405

9 Frohnert P P & Sheps S G Long term follow up study of periarteritis nodosa Amer J Med 43 8-14 1967

10 Hamrin B Jonsson N & Landberg T Arteritis in polymyalgia rheumatica Lancet I 397-401 1964

11 Harvey A Shulman L E Tumulty P A Conley C L & Shoenrich E H Systemic lupus erythematosus *Review of the literature and clinical analysis of 138* cases Medicine 33 291-437 1954

12 Hench P S Kendall E C Slocumb C H & Polley H F Effect of hormone of adrenal cortex (compound E) and of pituitary adrenocorticotropic hormone on rheumatoid arthritis Proc Mayo Clin 24 181-97 1949

13 Hench P S Kendall E C Slocumb C H & Polley H F Effects of cortisone acetate and pituitary ACTH on rheumatoid arthritis rheumatic fever and certain other conditions Arch intern Med 85 545-666 1950

14 Hollenhorst R W Brown J R Wagener H P & Shick R M Neurologic aspects of temporal arteritis Neurology 10 490-98 1960

15 Hoyle C Smyllie H & Leak D Prolonged treatment *of pulmonary sarcoidosis with corticosteroids Thorax* 22 519-24 1957

16 James D G Carstairs L S Trowell J & Sharma O P Treatment of sarcoidosis Lancet I 526-28 1967

17 Jessar R A Laurent Havers R W & Ragan C Natural history of lupus erythematosus disseminatus Ann *intern Med 38 717-31 1953*

18 Klemperer P Pollack A D & Baehr G Pathology of disseminated lupus erythematosus Arch Path 32 569-631 1941

19 Kogstad O A Polymyalgia rheumatica and its relation to arteritis temporalis Acta med scand 178 591-98 1965

20 Mulder D W Winkelmann R K Lambert E H Diessner G R & Howard F M Steroid therapy in patients with polymyositis and dermatomyositis Ann intern Med 58 969-76 1963

21 Pearson C M Patterns of polymyositis and their responses to treatment Ann intern Med 59 827-38 1963

22 Pollak V E Pirani C L & Kark R M Effect of large doses of prednisone on the renal lesions and life span of patients with lupus glomerulonephritis J Lab *clin Med 57 495-511 1961*

23 Report to the Medical Research Council by the Collagen Diseases and Hypersensitivity Panel Treatment of polyarteritis nodosa with cortisone Results after one year Brit med J 1 608-11 1957

24 Report to the Medical Research Council by the Collagen Diseases and Hypersensitivity Panel Treatment of polyarteritis nodosa with cortisone Results after three years Brit med J 1 1399-1400 1960

25 Rose A L & Walton J N Polymyositis A survey of 89 cases with particular reference to treatment and prognosis Brain 89 747-68 1966

26 Rosenthal A S & Thomison J B Alterations in the histopathology of systemic lupus erythematosus with steroid therapy Arthr and Rheum 7 502 11 1964

27 Sharma O P Colp C & Williams M H Course of pulmonary sarcoidosis with and without corticosteroid therapy as determined by pulmonary function studies Amer J Med 41 541-51 1966

28 Silzback L E Sarcoidosis Clinical features and management Med Clin N Amer 51 483-502 1967

29 Soffer I J Treatment of lupus erythematosus with steroids In Inflammation and diseases *of connective* tissue L C Mills & J H Moyer eds pp 643-48 Saunders Philadelphia & London 1961

30 Stone D J & Schwartz A A long term study of sarcoid and its modification by steroid therapy Amer J Med 41 528-40 1966

31 Wagener H P & Hollenhorst R W The ocular lesions of temporal arteritis Amer J Ophthal 45 617-30 1958

Acta med scand Suppl 500 pp 35–41 1969

GLUCOCORTICOIDS IN HAEMATOLOGY

by
Aage Videbæk

The use of glucocorticoids in haematology started about 20 years ago without any rationale according to the principle —we should try glucocorticoids because who knows they may help Nonetheless this led to many very interesting clinical results Since then attempts have therefore, been made to explain that there was in fact a very good reason to use these hormones in this or that condition However in many cases the available explanations are very superficial or even without basis and it is often necessary merely to acknowledge that the effect is a purely clinical experience and that over and above this it is necessary to relinquish the satisfaction of understanding why and how the effect is achieved

First a summary will be given of the action of corticosteroids on the blood and haematopoietic organs

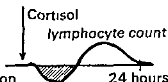
The lymphocyte count is affected within a few hours of a single dose with the appearance of an obvious but transient lymphocytopenia (7) which is followed by lymphocytosis and a return to normal values after 24 hours (fig 1) This rapid reaction is due to destruction of lymphoid tissue and thus lymphocytolysis which is manifest in disintegration of nuclei pyknosis and karyorrhexis Continued administration of glucocorticoids leads to moderate lymphocytopenia and furthermore to involution of the thymus spleen and lymph nodes all of which are however restituted within a few days of the withdrawal of treatment (6) All this is especially true of the so-called small lymphocytes

Neutrocytophilia occurs immediately after the administration of corticosteroids because of the release of a number of neutrophils from the so-called marginal granulocyte pool which normally comprises over half of the leucocyte population This effect is transitory and is responsible for the first upward slope of the graph (fig 2) in addition there is a reduction in the migration of granulocytes from the blood stream (44) If the administration of corticosteroids is continued there is a gradual increase in neutrophils during the first week reaching a maximum at twice or three times the original level after about three weeks After withdrawal normal values are not reached for about one week (25) In this case there is thus a more permanent overproduction of neutrocytes through an apparently direct influence on the bone marrow (47)

GLUCOCORTICOID EFFECT

Lymphatic tissue

Lymphocytopenia loss of cytoplasm
nuclear pyknosis
nuclear disintegration
reduced production



Tissue reduction

involution of thymus
- spleen
- lymph nodes
- lymphatic plaques

Fig 1

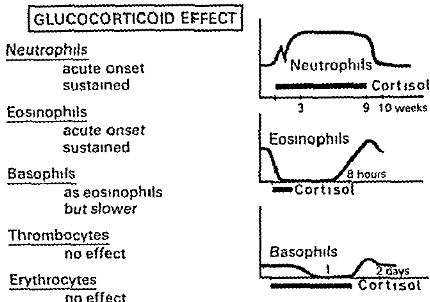


Fig 2

The eosinopenia induced by the corticosteroids which forms the essential part of Thorn s test is generally recognized but the mechanism behind the eosinopenia is not understood It is however known that the emission of eosinophils from the bone marrow is inhibited (21) There is a great deal of evidence which suggests that the eosinopenia is due to the reduction of circulating histamine induced by the corticosteroids (2a) and that this inhibits the release of eosinophils from the bone marrow (1) On more protracted treatment there is also inhibition of the production of eosinophils in the bone marrow (38) The number of *basophil* granulocytes also decreases although later than the eosinophils but 40 mg prednisone daily reduces the number to zero within about three days and 100 mg does so within about 24 hours (26)

Thrombocytes The corticosteroids neither increase the production of platelets nor extend the lifespan of the thrombocytes (42)

Erythrocytes It is improbable that there is any stimulation of erythropoiesis in normal individuals (31 40) and there is no induction of reticulocytosis nor change in the lifespan of the erythrocytes There would seem to be no experimental evidence of a direct effect of the corticosteroids on erythropoiesis in normal individuals The mechanism behind the moderate erythrocytosis in Cushing s syndrome is not understood By contrast both reticulocytosis and increase in haemoglobin may occur in many disorders including rheumatoid arthritis (15 22) but erythrocytosis does not occur on continued treatment

The phagocytotic ability is obviously compromised by corticosteroids this is true of both the neutrophils (19 39) and the entire reticulo-endothelial system (33)

The capillary effect is of considerable haematological interest The permeability is reduced leading to a reduction in the permeability of the capillary wall to 1) erythrocytes resulting in the possibility of the absence of haemorrhagic manifestations such as petecchiae and purpura despite the presence of severe thrombocytopenia 2) leucocytes of all types manifest in the fact that the so-called inflammatory exudate which is best observed in a so-called skin window becomes poorer in both polymorphonuclears and monocytes (44) this is of fundamental importance for the course of the inflammatory reaction

This brief review contains information of great importance to the use of glucocorticoids in the treatment of haematological disorders and malignant systemic disorders and also something about the risk associated with long term administration in the depressive effect on the lymphoid tissues on phagocytosis and the inflammatory exudate and in contrast to this the stimulant effect on neutrocytopoiesis and the reduction in capillary permeability

The main indications for the use of glucocorticoids in haematology will be discussed in brief

INDICATIONS FOR CORTICOSTEROID THERAPY IN THROMBOCYTOPENIA

ITP in children 80% well in 1 3 months
if not splenectomy
very few still show thrombocytopenia

ITP in adults Few recover
many require splenectomy
fewer helped by splenectomy
maintenance therapy often
small dosage
possibly intermittent

Symptomatic thrombocytopenia especially in
L E D Leukaemia
Myelomatosis etc
permanent or intermittent therapy

Fig 3

IMMUNE HAEMOLYTIC ANAEMIA

Previously *auto immune haemolytic anaemia* was often an extremely serious condition as only about one fifth of the patients went into spontaneous remission but corticosteroid therapy has changed the picture so completely that prednisone is now the drug of choice as it is effective in about 80% of cases caused by the so-called warm antibody and the mortality rate is less than 10% There are a large number of substantiated reports of this effect both clinical (5 8) and experimental (18) Thus NZB mice often develop a Coombs positive auto immune haemolytic condition Treatment with cortisone acetate acted both prophylactically in inhibiting the development of the anaemia and in those cases in which anaemia manifested itself it brought the anaemia to an end and the positive Coombs test became negative However the fly in the ointment was the fact that a large number of the mice which were treated with cortisone died of infection so that the nett gain was nil

There is no convincing evidence of the superiority of any one of the corticosteroids as compared with the others prednisone now reigns almost supreme

Prednisone reduces the concentration of the erythrocyte sensitizing antibodies in the blood (12) and in many patients the Coombs test becomes negative although this is not invariably the case despite the fact that the haemolysis is brought to an end This would suggest that the point of attack of the steroid is in all events not only the antibody production or the attachment of the antibody to the erythrocytes but it is probable that the haemolysis of the sensitized erythrocytes is counteracted by an inhibition of the function of the RES particularly in the spleen or outside this for example in the liver Splenectomy should not therefore be considered as indicated unless it becomes apparent that it is necessary to employ high doses of corticosteroids for a long period

THROMBOCYTOPENIA

It has proved difficult to demonstrate any sensitizing and agglutinating antibody in *idiopathic thrombocytopenia (ITP)* However it has been ascertained that typical ITP is immunologically determined (20) In such cases treatment with corticosteroids is the correct therapy as in this manner it is possible to achieve a reduction in the haemorrhagic diathesis regardless of the platelet count (23) furthermore the treatment often increases the count by counteracting the sequestration of the sensitized platelets in the spleen and other parts of the RES (43) It is conceivable that the treatment also makes it possible for the platelets which are formed after the beginning of treatment to ensure normal haemostasis even when the platelet count is only slightly increased (1 a) In addition corticosteroids reduce the serum con

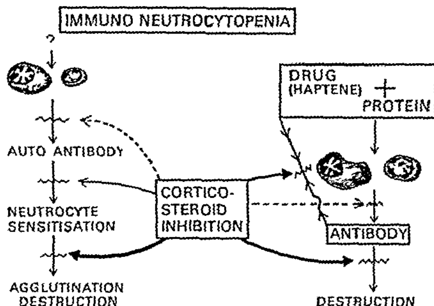


Fig. 4

centration of platelet antibody (20) and inhibit the reaction of agglutinin with the platelets (13)

There is such great individual variation in the spontaneous course of the disorder that evaluation *of the results of treatment is extremely difficult* The prognosis without treatment is particularly good in children in about 80 % of whom there is remission in the course of one to three months (41) Despite this ITP in children is a clear indication for corticosteroid therapy as such *hormone therapy nearly always brings the haemorrhagic tendency promptly* to an end (17a) despite the fact that the platelet count does not necessarily return to normal until a few weeks later at which time it is possible to reduce the dose of prednisone In this situation there is *little risk involved in the use of prednisone for such a short period In the remaining 20 % of children* splenectomy is carried out under cortisol cover and the majority of these patients are able to manage without the drug after a short time

The treatment of ITP in adults is similarly initially with corticosteroids again *particularly on the indication of haemorrhagic diathesis rather than to bring* the platelet count back to normal although there is an increase in the count in about 60 % of cases (3) In some patients – although this is a smaller proportion than in children – the treatment results in cure but *in others it is necessary to continue the treatment* and it is here that the question of splenectomy arises There is general agreement about the effect of corticosteroids until this period (34) However in many adults there is unaltered thrombocytopenia despite splenectomy and this necessitates intermittent or permanent therapy with corticosteroids the dosage may in periods be very small There are a *number of convincing reports that in some cases* ACTH may be effective when prednisone fails (1 11 45) or vice versa that corticosteroids may be effective when ACTH has no effect (11)

The corticosteroids are similarly of importance in *the treatment of secondary thrombocytopenia* not *least in systemic malignant diseases in which they* often permit the use of more intensive chemotherapy

AGRANULOCYTOSIS

It is definitely worth mentioning the place of the corticosteroids in the treatment of agranulocytosis as there are very few publications dealing with the clinical importance of this therapy A large number of investigations have resulted in the placing of the responsibility for the development of agranulocytosis on antibodies *(complete or incomplete) which cause an agglutination or destruction of leucocytes* This again leads to the rapid disappearance of the latter from the blood (30) apparently particularly in the lungs but it is thought that the antibodies can also affect the precursors in the bone marrow *resulting in the cessation of the production of neutrophils* The antibodies are often auto-antibodies which are known especially from a number of the connective tissue diseases but which also occur

more rarely, in malignant systemic diseases (27) As regards drug induced agranulocytosis the majority of workers consider that the leucocyte agglutination is caused by a complex consisting of the protein bound drug which acts as a haptene and its antibody (27, 32) Theoretically corticosteroids will be effective because 1) they inhibit the sensitization of the neutrophils with antibody (28 46) 2) the effect of the drug protein complex is inhibited (theoretically) and 3) the production of neutrophils is increased (15 22) (fig 4)

Surprisingly little concrete is known about the true value of corticosteroids in immune agranulocytosis In the period before the introduction of antibiotics the mortality rate was very high being 84% in Plum s amidopyrine material (37) but the mortality rate was considerably reduced when the stream of antibiotics started There are however a number of single observations from which the effect of corticosteroids is clearly apparent (10 28 32) The effect is apparently marked when leucocyte antibody is an associated factor whilst prednisone was completely without effect in 16 cases of agranulocytosis induced by chlorpromazine without any demonstrable agglutinins (35) and it was furthermore shown that chlorpromazine may act selectively and dose-dependently by inhibiting the nucleic acid synthesis of the neutrophils (36)

LEUKAEMIA

The involution of lymphoid tissue and disintegration of the circulating lymphocytes which has been demonstrated particularly in animals (6) naturally led to an investigation of the effect of corticosteroids in malignant systemic diseases of the lymphoreticular system but it rapidly became apparent that the main indication was stem-cell leukaemia However as thrombocytopenia and immune haemolytic anaemia are very common complications of the other leukaemias particularly chronic lymphogenous leukaemia the corticosteroids have a range of indications in the treatment of such complications They are also indicated if there are disturbing general symptoms which do not respond to other therapy

ACTH was used in *stem cell leukaemia* in children as early as in 1949 and since then a very large material of experience has been accumulated This demonstrates convincingly the beneficial effect of corticosteroids in stem-cell leukaemia first and foremost in children and of these in children with acute lymphogenous leukaemia (see among many others 16 24) Thus prednisone alone is capable of inducing complete remission in nearly 60% of children with lymphogenous leukaemia The higher the initial platelet count and the lower the initial leucocyte count the more easily the remission is achieved It has also been demonstrated that the remission produced by prednisone can be extended by 6-mercaptopurine (17) Iversen (24) has insofar as I have been able to ascertain demonstrated more conclusively than any one else that a higher proportion of and longer lasting remissions are produced by the combination of prednisone with 6-mercaptopurine and methotrexate than by prednisone alone

It is open to debate whether it is justifiable to compare the survival times in treated groups with the survival times of untreated groups of patients who suffered from the disorder at a time different from that of the treated groups There is nevertheless agreement that before 1948 remission was exceptional (48) and a duration of illness of more than one year very rare (49) whereas now by contrast remission is the rule and the survival time has become obviously longer (2)

DISCUSSION

Dr Loren en For the first time in this symposium we have heard about some groups of diseases in which the glucocorticoids seem to have convincing effect on the mortality rate namely immune haemolytic anaemia idiopathic thrombocytopenic purpura and stem-cell leukaemia

Dr Nissen It has been stated that in ITP the glucocorticoids inhibit the sequestration of platelets in the spleen Is any thing known about the mechanism of this?

Dr Harvald With reference to drug induced agranulocytosis is it true that one definite compound for example methyl thiouracil or propyl thiouracil may lead to a direct toxic inhibition of leucopoiesis or may it occasionally act as a haptene and induce the formation of antibodies against the complex?

Dr Rossel When it is known that the capillary resistence is increased during steroid therapy how can one explain the petecchiae seen during steroid treatment of rheumatoid arthritis?

Dr Videbæk With regard to the sequestration of thrombocytes in the spleen I can put forward no other explanation than the fact that in patients with idiopathic thrombocytopenia the thrombocyte life span is increased during prednisone therapy I cannot say whether this increase is in fact due to the reduction in the sequestration in the spleen or whether the cause is an inhibition of immunological reactions However in some of these patients it has been observed *that the life span of the thrombocytes is increased after* splenectomy as an expression of the fact that some part is

played by splenic factors and in this respect it is first and foremost sequestration which is implicated

It is difficult to answer the question about the haptene effect of such compounds as MTU and PTU because of the great technical difficulties associated with the demonstration of leucocyte and thrombocyte agglutinins If investigations are carried out very soon after the onset of the agranulocytosis it is in many cases possible to demonstrate leucocyte agglutinins this corresponds to the finding that in such cases the effect of the glucocorticoids is very pronounced The haemorrhagic tendency in patients with rheumatoid arthritis and other patients who are receiving long-term therapy with prednisone is quite a different phenomenon This is due to a weakening of the capillary wall consequent to the action on the connective tissue in the vascular wall The capillary permeability is not increased

Dr Bennike Is there any indication for steroid therapy in patients with chronic leucopenia? Some of these patients are troubled by recurrent attacks of stomatitis and it is only possible to keep them free from symptoms by permanent steroid therapy

Dr Videbæk Chronic neutrocytopenia can be an indication It is however a question of diagnosis In many of those patients it is possible to demonstrate nuclear antibodies or leucocyte agglutinins A number of the chronic granulocytopenias are manifestations of other diseases e g SLE Quite a different problem is when an agranulocytosis or a neutrocytopenia should be considered of importance It is my impression that a neutrocytopenia is not considered to be so serious nowadays as it was previously Otherwise there is far too little differentiation between granulocytes and lymphocytes in this respect It is presumably far more serious to suffer from severe lymphocytopenia than agranulocytosis

Dr Lorenzen With regard to idiopathic thrombocytopenia in children which has a good spontaneous prognosis Should children with idiopathic thrombocytopenia in whom there is no evidence of haemorrhagic diathesis always be treated on the principle that haemorrhagic complications can occur or is it permissible to wait and see? Is the incidence of haemorrhagic complications of this disease in children so high that treatment should be instituted under all circumstances

The effect of this treatment in adults is by no means pronounced Is it known whether the mortality rate has in any way been altered by steroid therapy despite the lack of change in the thrombocyte count

Dr Videbæk The crucial factor in the decision to treat ITP in children is the extent and severity of the haemorrhagic diathesis and not so much the platelet count In adult patients there is adequate documentation of the fact that corticosteroids extend the life-span by a reduction of the haemorrhagic complications

REFERENCES

1 Archer R K Eosinophil leucocytes and their reactions to histamine and 5-hydroxytryptamine J Path Bact 78 95–103 1959

1a Bonnin J A The management of thrombocytopenic states with particular reference to platelet thromboplastic function Brit J Haemat 7 250–60 1961

2 Burchenal J H Long term remissions in acute leukemia Spontaneous and induced Series haematologica 1 47–56 1965

2a Code C F & Mitchell R G Histamine eosinophils and basophils in the blood J Physiol (Lond) 136 449–68 1957

3 Cohen P & Gardner F H The thrombocytopenic effect of sustained high-dosage prednisone therapy in thrombocytopenic purpura New Engl J Med 265 611–617 1961

4 Dacie J V The haemolytic anaemias Part II p 682 Churchill Ltd London 1962

5 Dameshek W & Komninos Z D The present status of treatment of autoimmune hemolytic anemia with ACTH and cortisone Blood 11 648–64 1956

6 Daughaday W H Williams R H & Daland Geneva A The effect of endocrinopathies on the blood Blood 3 1342–66 1948

7 Daugherty T F Adrenal cortical control of lymphatic tissue mass In F Stohlman (ed) The Kinetics of cellular proliferation p 264–74 Grune & Stratton New York, 1959

8 Dausset, J & Colombani, J The serology and prognosis of 128 cases of a i h a Blood 14 1280–1301 1959

9 Gasser C & Anderes R Cortison und ACTH bei Thrombopenien im Kindersalter Schw med Wchnschr 83 1036–37 1953

10 DeGruchy G C Haematology in medical practice 2nd ed p 342 Blackwell Oxford 1964

11 Eisen M J & Ruchnikoff Isadore The treatment of idiopathic thrombocytopenic purpura with ACTH and cortisone N Y St J Med 53 1456–58 1953

12 Evans R S Bingham Margaret & Turner Elisabeth Autoimmune haemolytic disease Observations of serological reactions and disease activity Ann N Y Acad Sci 124 422–440 1965

13 Falconer E H Schumacher J C Purpura haemorrhagica due to ingestion of sedormid Experimental observations and report of a case Arch intern Med 65 122–38 1940

14 Faloon W W Greene R W & Lorner Eugene L The haemostatic defect in thrombopenic purpura as studied by the use of ACTH and cortisone Amer J Med 13 12–20 1952

15 Finch C C Crockett Ch J Ross J F Bayles T B Hematologic changes with ACTH and cortisone therapy of rheumatoid arthritis Blood 6 1034–50 1951

16 Freireich E J & Frei, E III Recent advances in acute leukemia In Moore C V & Brown E B (eds) Progress of Hematology vol IV pp 187–202 Grune & Stratton New York 1964

17 Freireich E J and 14 others The effect of 6-mercaptopurine on the duration of steroid induced remission in acute leukemia A model for evaluation of other potentially useful therapy Blood 21 699–716 1963

18 Giltenan P J Holmes M C & Burnet, F M Cortisone acetate treatment of haemolytic anaemia in NZB mice Aust J exp Biol med Sci 43 523–32, 1956

19 Greendyke R M Bradly E & Swisher S N Studies on the effect of administration of ACTH and adrenal

corticosteroid on erythrophagocytosis J clin Invest 44 746-56 1965

20 Harrington W J Minnich Virginia & Arimura Grace The autoimmune thrombocytopenias In Tocantins L M (ed) Progress in Hematology vol I pp 166-192 1956

21 Hudson G The marrow reserve of eosinophils Brit J Haemat 10 122-30 1964

22 Hävermark N G & Nordenson N G Further studies on blood and bone marrow after administration of ACTH and cortisone Acta haemat (Basel) 9 227-37 1953

23 — — The effect of ACTH and cortisone on haemorrhagic diathesis with thrombocytopenia Acta haemat (Basel) 9 107-14 1953

24 Iversen T Leukaemia in infancy and childhood Thesis Munksgaard Copenhagen 1966

25 John T J Leukocytosis during steroid therapy Amer J Dis Child 111 68-70 1966

26 Juhlin L The effect of corticotrophin and corticosteroids on the basophil and eosinophil granulocytes Blood 29 157-65 1963

27 Killmann S Å Leukocyte agglutinins Thesis Blackwell Oxford 1960

28 Moeschlin S Leukocyte autoantibodies Acta haemat (Basel) 20 167-78 1958

29 Moeschlin S Siegenthaler W Gasser C & Hässig A Immunopancytopenia associated with incomplete cold hemagglutinins in a case of primary atypical pneumonia Blood 9 214-226 1954

30 Moeschlin S & Wagner K Agranulocytosis due to the occurrence of leukocyte agglutinins Acta haemat (Basel) 8 29-41 1952

31 Morrison J H & Toepfer J R Cellular changes in the bone marrow following chronic treatment of rats with cortisol Acta haemat (Basel) 38 250-54 1967

32 Müller W Zur Pathogenese der Arzneimittelagranulocytose Acta haemat (Basel) 12 304-21 1954

33 Nicol T & Bilbey D L J The effect of various steroids on the phagocytic activity of the reticuloendothelial system In John H Heller (ed) Reticuloendothelial structure and function pp 310-20 Ronal Press Co New York 1958

34 Norcross J W & Cyr D P Treatment of chronic idiopathic thrombocytopenic purpura in the adult Post grad Med 33 74-79 1963

35 Pisciotta A V Ebbe S Lennon E J Metzger G O & Madison F W Agranulocytosis following administration of phenothiazine derivatives Amer J Med 25 210-23 1958

36 Pisciotta A V & Kaldahl Joyce Studies on agranulocytosis IV Blood 20 364-76 1962

37 Plum P Clinical and experimental investigations in agranulocytosis Lewis & Co London 1937

38 Quittner H Wald N Sussmann L N & Antopol W The effect of massive doses of cortisone on the peripheral blood and bone marrow of the mouse Blood 6 513-21 1951

40 Reisner E H Effect of steroid hormones on hemopoiesis in vitro Blood 27 460-69 1966

41 Shulman I Management of idiopathic thrombocytopenic purpura Pediatrics 33 379-82 1964

42 Shulman N R Marder N Hiller M C & Collier E M Platelet and leukocyte isoantigens and their antibodies Serologic physiologic and clinical studies In Moore C V & Brown E B (eds) Progress in Hematology vol IV pp 222-304 1964

43 Shulman N R Marder V J & Weinrach R S Similarities between known antiplatelet antibodies and the factor responsible for thrombocytopenia in idiopathic purpura Ann N Y Acad Sci 124 499-542 1965

44 Speirs R S & Wenck U Effect of cortisone on the cellular response during allergic inflammation Acta haemat (Basel) 17 271-279 1957

45 Stefanini M Perez, Chatterjea J B Dameshek W & Salomon L Corticotrophin (ACTH) and cortisone in idiopathic thrombocytopenic purpura J Amer med Ass 149 647-53 1952

46 Steffen C Fessel F & Schindler H Untersuchung über den Einfluss von Cortison auf den Ablauf von Antigen Antikorper und Autoantikorperreaktionen Acta haemat (Basel) 17 81-98 1957

47 Strausz I Kékes E & Szebeni Agnes Mechanism of prednisolone induced leukocytosis in man Acta haemat (Basel) 33 40-48 1965

48 Southam C M Craver L F Dargeon H W & Burchenal J H A study of the natural history of acute leukemia with special reference to the duration of the disease and the occurrence of remissions Cancer 4 39-59 1951

49 Tivey H Prognosis for survival in the leukemias of childhood Pediatrics 10 48-59 1952

Acta med scand Suppl 500 pp 43–48 1969

GLUCOCORTICOIDS IN ALLERGIC DISEASES

by
Michael Schwartz

The logical treatment of allergic diseases and allergic reactions in general – to remove the responsible allergen from the patient or the patient from the allergen – is just as correct in theory as it is difficult or impossible to achieve in practice

In some cases it is possible to accustom the body to contact with the compounds against which usually for unknown reasons it has formed antibodies by means of the so-called desensitization therapy and in this way to prevent or weaken the allergic reaction There are however large gaps in our understanding of this treatment and in the majority of allergic diseases the results are very poor It is necessary to acknowledge that so little is known today about the allergic diseases that in by far the majority of cases the treatment is expectant and purely symptomatic The situation in the allergic diseases does not therefore differ greatly from that in the other disorders which are discussed in this symposium

The allergic diseases which will be discussed in the following are

Hay fever
Bronchial asthma
Vasomotor rhinitis
Besnier s prurigo
} *the atopic disorders*

These disorders are presumably due to a common genetic constitution and have many features in common (6)

To these may be added
Urticaria and angioneurotic oedema (Quincke)
Allergic reactions to drugs
Allergic shock
Allergic skin diseases
Gastro intestinal allergy

Finally it is probable that a number of the other disorders and reactions which have been discussed in this symposium are due to antigen antibody reactions (certain blood disorders certain liver diseases renal and gastro intestinal diseases) but these are traditionally dealt with under the organs concerned

The use of the glucocorticoids in allergic disease began in 1949 as soon as cortisone (and ACTH) was introduced Today – and like Dumas we can say 20 years after – a considerable body of experience has been accumulated concerning the practical clinical applications of these agents in allergic disease But unfortunately the hope which was raised of obtaining a better aetiological and especially pathogenic understanding of these disorders through the action of the corticosteroids has been disappointed

Today – as was the case 20 years ago – we still speak of the anti allergic action which is very considerable of the glucocorticoids without knowing what it is we really mean In contrast to the antihistamines the glucocorticoids have no measurable effect on the urticarial cutaneous reaction which is perhaps manifest most typically in hay fever and they similarly do not affect the allergic antibodies (reagins) in the blood On the other hand the corticosteroids are most effective in asthma in which the effect of the antihistamines approximates to zero

It is more reasonable to consider that the often dramatic action which may be achieved with the glucocorticoids in allergic diseases is associated with the anti inflammatory effects of the agents Thus the glucocorticoids have a marked suppressive effect on the antigen antibody reaction of the type delayed hypersensitivity It is then another question of how much is in fact known about the anti inflammatory action

One very important feature of the effect of the glucocorticoids on allergic diseases and reactions – of whatever type these may be – is that there is *no* question of an instantaneous effect Regardless of whether the patient suffers from asthma vasomotor

rhinitis or eczema there is always an interval of up to ten hours following the administration of a full therapeutic dose before the effect begins to be manifest This is found regardless of the mode of administration (3) There is thus nothing to be gained by using intravenous injections of glucocorticoids in allergic (anaphylactic) shock where the treatment of choice is still epinephrine

There would seem to be no difference in the action of the various synthetic glucocorticoids in allergic disease The latest synthetic glucocorticoids are thus no better than prednisone which is used by the majority of physicians including the author Administration of the adrenocorticotropic hormone (ACTH) has the same effect as the steroids but for a number of reasons this form of treatment is no longer used very often The present writer, who can remember the very dramatic effect of ACTH in the early period when cortisone was in short supply will not be surprised if it should turn out that the steroid production which is induced by ACTH in the adrenals in some way or other is found to have a particularly marked anti allergic effect

GENERAL LINES IN THE TREATMENT OF ALLERGIC DISEASES WITH CORTICOSTEROIDS

As mentioned glucocorticoid therapy is generally not the treatment of choice in allergic diseases In many cases these disorders are quite transient and their course is so harmless that either no treatment or another form of symptomatic treatment is to be prefered

Steroid therapy is not curative but merely symptomatic and not unless the symptoms reach a considerable degree of severity should the use of these agents be contemplated

On the other hand many of the allergic diseases and reactions are often transient self limiting occurrences as for example many of the allergic reactions to drugs and serum sickness In these conditions there is no great danger that the patient would enter the more dangerous long term steroid therapy and in such cases it is not necessary to hesitate too long before initiating steroid therapy However it is important to be aware of the spontaneous course of these disorders in order to be able to withdraw the hormones as soon as possible after the spontaneous cure has occurred

Where the allergic disease is of a chronic nature as vasomotor rhinitis Besnier's prurigo (which is however usually treated with local applications) and that disorder which is of greatest importance asthma then the situation is quite different In all these disorders the glucocorticoids have an admirable symptomatic effect which especially in the case of asthma one could not do without nowadays On the other hand it should be stressed that the more effective such an agent is in a chronic disease the more difficult it is to withdraw the treatment Asthma patients are therefore, particularly common participants in the group of those receiving long term corticosteroid therapy, which involves all the risks which will be discussed later in this symposium

GLUCOCORTICOID THERAPY IN ALLERGIC DISEASES AND REACTIONS OF SHORT DURATION

Hay fever

By far the most common form in Denmark is due to grass pollen and lasts as a rule from a few weeks up to two months being partly dependent on local conditions

In severe manifestations in which conventional treatment with desensitization + antihistamines has proved inadequate it may be necessary to contemplate the use of glucocorticoids The principle is to give a moderately large dose initially e g prednisone 10 mg q i d for two days and thereafter rapidly to reduce the dose (over two to three days) to 5 mg q i d or less The dosage must be adjusted to the effect and it is useful to ascertain the pollen count in the air in order to determine the duration of treatment Here in Denmark the newspapers do *not give reports of the pollen counts but informa*tion obtained from other patients with hay fever may give guidance Another form of dosage for corticosteroid therapy in hay fever has become accepted during recent years the use of injections of crystalline suspensions Good results of the injection treatment of hay fever with methylprednisolone (80 mg per dose) have been reported from Denmark (5) It is possible for the patient to get through the season on very few injections

Bronchial asthma

Steroid therapy of the acute asthma attack and also of its most severe manifestation *status asthmaticus* is the most effective treatment available in this disorder The rule mentioned previously applies here

one should not use a sledge hammer to crack a nut In mild attacks other symptomatic therapy should always be tried first and often this will prove adequate If the attack does not disappear within a reasonable length of time the patient will often be admitted to hospital and if this in itself does not bring the attack to an end then steroid therapy may be considered

The principle behind the treatment is exactly the same as that in hay fever The most important points are to begin with a large dose and to keep the duration of treatment short In the acute attack and in *status* we usually employ the old fashioned ACTH therapy beginning with 60 units q i d and rapidly diminishing the dose with withdrawal after eight days

This standard treatment brings almost every attack of asthma to an end and has moreover the advantage that it also brings itself to an end A completely comparable self limiting course of steroids in acute asthma is recommended by the Medical Research Council in England 60 mg prednisone on the first day falling by 5 mg daily until the end of the course after 12 days (2)

Short courses of steroid therapy for asthma patients who are to undergo operations especially in the thorax generally lead to an improvement in the pulmonary function to the best which can be achieved in that particular patient

The contra indications to treatment are the usual peptic ulcers heart failure mental illness etc and as usual the contra indications are not absolute if treatment is really imperative

Urticaria and (allergic) angioneurotic oedema

In introduction it may be mentioned that the dominant inherited form of angioneurotic oedema which is due to an absence of the serum inhibitor of C^1-esterase has nothing to do with allergy and the effect of steroids in these cases is dubious

The most important thing in these disorders is to clarify the aetiology which frequently turns out to be a drug of one sort or another and the duration of the illness is therefore limited – if the cause is removed The principles of steroid therapy are the same as those in hay fever As a rule steroid therapy must be continued for at least one and often two weeks – but if at all possible no longer Month long courses of steroids in urticaria (so-called chronic urticaria) – the aetiology of which is unknown – should only exceptionally be necessary

The treatment of choice in urticaria (and allergic angioneurotic oedema) should be the antihistamines rather than the steroids

Serum sickness

This disorder is probably rare nowadays but prophylactic treatment with 1 ml tetanus antitoxin does lead to occasional cases being admitted to almost every medical department during any year The dominating symptoms are usually urticaria and angioneurotic oedema and they almost always disappear rapidly and quite spontaneously It would have to be an unusually severe case for the physician to be tempted into giving glucocorticoid therapy The lines to be followed are the same as in urticaria It will usually be found that just when the decision to use steroids has been taken the symptoms disappear of themselves

Allergic shock

Allergic (anaphylactic) shock is a state to be dreaded In by far the majority of cases it is the doctor himself who is an essential factor in the aetiology as he has prescribed or injected the agent which has produced the shock As mentioned above there is a considerable latent period before any effect of glucocorticoids is manifest in allergic conditions and although steroids may have a valuable therapeutic effect in the late sequelae of an antigen antibody reaction such as for example oedema following the use of serum it is not the steroids which should be considered in the first place It is epinephrine antihistamines and other symptomatic treatment of shock which are required If it is considered desirable to give glucocorticoids they can just as well be given orally in this situation To start treatment of allergic shock with steroids is to waste precious time

Allergic reactions to drugs

It is outside the framework of this symposium even to count up the many manifestations of drug allergy Many forms are dealt with elsewhere in the symposium for example those occuring in certain blood diseases liver diseases peri arteritis nodosa etc The most common are probably the dermatological manifestations and several of these especially exfoliative erythrodermia may be life-endangering

Once the diagnosis is established the problem – whether or not to use glucocorticosteroids – must be settled by the course of the illness Certain con

ditions – e g exfoliative erythrodermia – form absolute indications for treatment and are perhaps among the greatest triumphs attributable to steroid therapy Other cases such as those of quiet transient erythema are best treated expectantly possibly with calamine lotion If it is decided to give glucocorticoid therapy then full doses should be given e g 40–60–80 mg prednisone daily with a subsequent gradual slow and tentative reduction Naturally the drug suspected of giving rise to the condition must be withdrawn possibly all drugs if there is any doubt

These reactions are characteristically acute often of very dramatic appearance but as a rule transient The subjective disturbances itching burning in the skin erythema oedema etc may be considerable The effect of steroid therapy is generally very satisfactory and of rapid onset Most frequently it is possible to conclude therapy within a fortnight On the withdrawal of treatment there seems sometimes to be some excitement in the skin again but this disappears spontaneously without it being necessary to restart the treatment

The effect of steroids on allergic drug reactions is as mentioned above extremely satisfactory – on condition that the administration of the allergen ceases The effect is far more dubious if for some reason or other the administration of the drug to which the patient is sensitive is continued Similarly it is not to be anticipated that the glucocorticoids will have any particular prophylactic effect when they are given in the hope of permitting the use of drugs to which the patient has previously shown severe reaction This form of treatment is extremely dangerous and should only be attempted when it is a matter of life or death

LONG TERM THERAPY WITH GLUCOCORTICOIDS IN ALLERGIC DISEASES

Bronchial asthma

Asthma is by far the most important of the allergic diseases both with regard to incidence and to severity The use of glucocorticoids in the treatment of the acute attack has been described in brief above

Long term corticosteroid therapy of asthma should be considered when the illness does not permit the patient to lead a normal or nearly normal life and where other therapy has proved to be ineffective

All studies over the years have unanimously demonstrated the extremely satisfactory results of such treatment and there are by now such large groups of patients treated in this manner who have been observed for sufficiently long periods for it to be possible to give general and consistent directions for this treatment The experience gained over the years has proved to be quite uniform from one group of workers to the next (2 3 4 8)

Indications for long term steroid therapy in asthma

a) The first prerequisite before starting on a long term glucocorticoid treatment which may last for years or even throughout the patient s life is that conventional therapy has proved ineffective or else has had so little effect that the patient is severely handicapped by his illness If there is any doubt about this then the patient should be observed for a suitable period on conventional therapy

b) A second important consideration is whether steroid therapy will be effective in the particular patient concerned It is necessary to ensure that the action of the steroids is so marked that it is worth while taking the risks involved Useful information in this respect may be gained by ordinary clinical observation including stethoscopy, but it is better to employ more objective measurements of the pulmonary function for example examination of the F E V

c) The patient (and his doctor!) must understand the principle of the treatment especially that the steroids are not drugs to be taken against the acute attack in the same way as ephedrine spray etc The patient must follow the doctor s instructions and the doctor must be willing constantly to maintain contact with his patient Asthma patients on long term steroid therapy in whom there is sudden deterioration of the illness run the risk of the attack proving fatal within a few hours In serious deterioration of the illness – as may be seen especially in acute respiratory tract infections – the dose of steroids must immediately be increased to full strength (40–60 mg prednisone) and it is possible to save a great deal of time if the patient is aware of this It may take 24–36 hours before the steroid effect again reaches its height In more severe attacks these patients should always be admitted to hospital (4)

Duration of treatment

The final aim of the treatment is to be able to withdraw the steroid therapy Experience has shown that this is only rarely possible (about 10 % of patients)

and that it may represent a risk Maunsell et al (4) report that 11 out of 52 patients developed status asthmaticus in connection with attempts to withdraw steroids and three of them died In such cases in particular it is important to restart the treatment at once and not to try to treat the patient expectantly Patients with rheumatoid arthritis develop joint pain etc when steroids are withdrawn Patients with asthma develop bronchial constriction which may well prove fatal

Apart from this there is a certain degree of addiction associated with steroid therapy which makes it even more difficult to withdraw these drugs

Dosage of glucocorticoids

As in acute asthma the initial dose should be relatively large (40–60 mg prednisone in four divided doses) The dosage is then reduced over a few days to about 20 mg daily and attempts are made under the guidance of the effect to reduce it as far as possible by means of a slow and gradual diminution in dosage The majority of patients in whom it proves necessary to continue usually end with a dose of 5–15 mg prednisone daily It is undesirable to give larger doses and less than 5 mg daily approaches homeopathy although there are patients who say that they cannot do without their 2 5 mg every or every other, day A dose of steroids which keeps the patient completely symptom free is in general too large

Naturally, advantage should at the same time be taken of the effects of other drugs (theophyllamine spray etc)

It may be mentioned that even after many years of treatment the effect of the steroids would seem to remain constant It is unnecessary to fear that one will be obliged to increase the dose over the years (4)

Effect of treatment

On the whole it can be said that there is an excellent effect of long term steroid therapy in about 60 % of asthma patients some effect in about 25 % and therapeutic failure in only about 5 %–15 % of asthma patients

The effect is more or less uniform regardless of sex and age and also of the duration of the illness

It is hardly surprising that mild cases react more favourably than severe

It is of no consequence to the effect of the treatment whether the asthma is due to infection alone (intrinsic asthma) or whether there are also convincingly demonstrable external factors which provoke the attacks The risks of fatal asthma on attempts to withdraw therapy and particularly when it is not restarted in full dosage have been described above

Side effects of steroid therapy of asthma

This question is dealt with elsewhere in the symposium The incidence is given as about 20 % but must vary according to what are understood as side effects There would not appear to be any risk of increased tendency to infection even after a number of years of treatment and the other side-effects are those which are generally recognized Many asthma patients have now received treatment for 5–10 years or even longer

In children an extra complication which may be seen is retardation of growth After reduction of the dosage or withdrawal of the treatment the children are stated to catch up again Several authors state that it is necessary to be considerably more reluctant to give steroid therapy to children than to adults

Inhalation therapy with steroids

The writer has no personal experience of this form of treatment Despite the fact that theoretically there should be certain advantages of this method it has proved disappointing in the majority of trials The difficulty lies in among other things obtaining a particle size which is not so small as to give merely an absorption effect and not so large that the drops do not reach the bronchioli (1)

The conclusion must be that long term steroid therapy in asthma is here to stay Despite the by no means inconsiderable risks it must be accepted that in many cases this is the only form of treatment which is capable of enabling the patient to live a more or less normal life As long as we have so little knowledge about the true biochemical basis of asthma other allergic diseases – and the other diseases which have been discussed in this symposium – then it is necessary to be satisfied with symptomatic treatment and happy that such is available The price which must be paid – the side-effects – is not to be complained about – it is always expensive to be poor

Long term glucocorticoid therapy in other allergic diseases

Of the allergic diseases other than asthma in which the question of long term therapy may be raised

vasomotor rhinitis Besnier's prurigo (atopic dermatitis) chronic urticaria and the rare cases of gastro intestinal allergy may be named

The symptoms of these diseases must be very disturbing long lasting and quite intractable to other therapy before steroid treatment for long periods is considered

The treatment must follow the lines laid down for the treatment of asthma

It is possible to treat one particular form of gastro intestinal allergy characterized by severe eosinophilia and hyproproteinaemia due to gastro intestinal loss of protein very effectively with steroids If it is possible to demonstrate the allergen – which is often milk – then the elimination of this compound from the diet is curative This is another example of the fact that knowledge about the aetiology and pathogenesis of an illness is of greater importance to the patient than the availability of what is it is true often an outstanding symptomatic therapy

DISCUSSION

Dr Lorenzen Are there any reasons to be especially careful about treating the acute attack of bronchial asthma with glucocorticoids as it is known that the treatment will be of very short duration and may lead to a crucial improvement within a very short time?

Is there any investigation which has demonstrated that the lethality of the acute attack of asthma has been reduced since the introduction of the glucocorticoids?

In what way does long term glucocorticoid therapy affect the course of the illness? Is there any increase in the incidence of such complications as reduced pulmonary function bronchitis and pneumonia? Has the mortality rate altered as a result of long term therapy?

Dr Schwartz There has probably been little change in the lethality of asthma although in certain areas the mortality rate has increased for example an increase in mortality rate has recently been registered in England It was thought that the steroids were responsible for the increase but a very comprehensive investigation revealed that this is probably not the case

There is no increase in the complications of bronchial asthma in connection with steroid therapy On the contrary there would seem to be a reduction in the incidence of bronchitis Pneumonia is similarly not more common

I consider that there is a very real indication for treatment with glucocorticoids in patients with bronchial asthma because the patients feel better and have a far greater working capacity I am in complete agreement with you in that it is precisely because steroid therapy brings the patient out of the acute attack more rapidly that this is such a reasonable therapy But there is reason to observe the patient for a period before starting therapy Many attacks of asthma disappear spontaneously soon after the patient has been admitted to hospital

Dr Bruun On the whole I agree with Schwartz, but I would like to make some comments on long term therapy

The percentage of patients with bronchial asthma in whom long term therapy with steroids is necessary has been found in most parts of Europe and the USA to be in the region of 8 16%. Where the dosage of the glucocorticoids in long term therapy is concerned it is usually possible to manage with very small doses e g 5 7 ½ mg prednisone daily This is of great importance as it means that there is a sharp decrease in the incidence of side-effects

Dr Videbæk Is there any evidence that corticotrophin is more effective than the glucocorticoids in asthma?

Dr Schwartz No not insofar as I am aware

Dr Andersson Some workers consider that the remission following corticotrophin therapy is of longer duration than that after the glucocorticoids Moreover the inhibitory action of the glucocorticoids on the adrenal cortical function is avoided For these reasons I consider that it is an advantage to retain corticotrophin therapy

Dr Kjerulf May I enquire about the value of using the eosinophilia in the peripheral blood as a parameter in establishing the indications for initiating therapy and for its maintenance I agree with Schwartz that it is first and foremost the frequency and severity of the attacks which indicate treatment The question is whether it is possible to decide from the number of eosinophil leucocytes in the peripheral blood whether the patient is about to develop an attack Should one carry out regular eosinophil counts?

Dr Schwartz There is no doubt that in asthma patients with very severe eosinophilia there are very frequently indications for steroid therapy The fluctuations in their eosinophilia and asthma run in parallel so that it is possible to treat them according to either their symptoms or their eosinophilia with the same results

REFERENCES

1 Annotation Aerosol Steroids in Bronchial Asthma Lancet I 1412 1413 1963
2 Editorial Corticosteroid Therapy in Asthma Brit med J I 1413 1414 1961
3 Lowell F C *Allergic disorders* In *Clinical uses of adrenal* steroids ed Brown J & Pearson Carl M pp 152 161 447 McGraw Hill Book Co New York 1962
4 Maunsell K Bruce Pearson R S & Livingstone J L Long term corticosteroid treatment of asthma Brit med *J I 661–665 1968*
5 Pallesen A E Behandling af høfeber med steroiddepot præparater Depo Medrol (Metylprednisolon) Ugeskr Læg 573 576 128 1966
6 Schwartz, M Heredity in Bronchial Asthma Thesis *Munksgaard Copenhagen p 288 1952*
7 Walsh S D & Grant J W B Corticosteroids in treatment of chronic asthma Brit med J II 796 802 1966

Acta med scand Suppl 500 pp 49-54 1969

GLUCOCORTICOIDS IN LIVER DISEASES

by
Flemming Quaade

When one tries to survey the present therapeutic front against hepatic disease it is easy to be pessimistic However this feeling is only justified with a number of important reservations The improved therapeutic measures of the past few years have in fact also benefited patients with liver disease One need only mention the advances in the rational treatment of hepatic coma in control of bleeding oesophageal varices and the establishment of portocaval anastamoses the improved diuretic therapy of oedema and ascites and the treatment of the haemodynamic renal failure in cirrhosis

There is therefore much cause for satisfaction but unfortunately optimism is appropriate only with regard to the culmination of liver disease — coma — or to the complicating phenomena haemorrhage from varices and water retention In spite of all efforts there is still no prophylaxis nor any causal therapy of hepatic diseases and this is true of both the acute and chronic forms It is still impossible to culture the virus of acute hepatitis and therefore also impossible to produce any vaccine against it and up to the present we have also been unable to prevent or cure chronic hepatitis and cirrhosis of the liver This unfavourable situation explains why the corticosteroids were greeted with hope in liver disease as in almost all other serious diseases It must however be emphasized that the use of the steroids was not a leap in the dark but justified by a certain rationale The production of the adrenal cortical hormones raised the possibility of attacking some of the fundamental processes involved in liver diseases as it was demonstrated that cortisone inhibits the inflammatory reaction and formation of fibrosis More specific and promising was the ability of the glucocorticoids to lower a raised serum bilirubin The first explanation of this finding that the steroids have a choleretic effect is probably incorrect as the steroids do not increase the flow through a drain in the biliary passages The most likely hypothesis at the present moment is that the steroids lead the break down of bilirubin to unpigmented metabolites

Great hopes were also attached to the immunosuppressive action of the steroids after it became apparent that the liver might be affected in systemic collagen disease and that some patients with pure hepatic diseases showed evidence of autoimmunization

In the following an attempt will be made to review the achievements of corticosteroid therapy in the most important hepatic disorders In this connection it should be mentioned that by glucocorticoids are meant the synthetic fluor free preparations which are nowadays universally preferred and which in the following will for the sake of simplicity be termed steroids'

The liver diseases to be considered here belong to the large groups which must for the present be called primary In these diseases the liver is the only organ affected or at any rate it occupies a central position in the clinical picture These groups include the so-called lupoid hepatitis despite the fact that this is part of a collagen disease Secondary liver diseases in which the organ is affected by metastasizing cancer and the so-called storage diseases such as the glucogenoses lipoidoses and haemochromatosis are outside the scope of this discussion This leaves the following main groups to be considered

acute hepatitis
chronic hepatitis
cirrhosis of the liver

It is well known that *acute virus hepatitis* may vary greatly in severity and duration especially from one epidemic to the next and with the age and sex of the patient It should however be borne in mind that the great majority of cases will recover completely and spontaneously Everybody agrees that

the steroids may have an excellent effect on some of the manifestations of acute hepatitis the appetite is improved and the subjective sense of illness allevi ated there is a rapid fall in the serum bilirubin and to a lesser extent in the raised transaminase levels *On the other hand the steroids do not affect the* electrophoretic pattern the alkaline phosphatases the histological picture nor the duration of the ill ness (4 6 11)

The conclusion must be that steroid therapy is not justified in the routine treatment of a disease which has such a good spontaneous prognosis as that usual in acute hepatitis It may however occasion ally be justifiable to administer steroids to some patients especially post menopausal women with one or more of the following poor general condi tion with nausea recurrent exceptionally deep and persistent icterus or fulminant course threatening to deteriorate into acute yellow atrophy Unfortu nately in these dangerous types the steroid effect is not convincing (2 6 7 13) Once it is decided to use steroids in acute hepatitis their admini stration must be continued well into the con valescent period as early withdrawal is often fol *lowed by relapse*

Evidence is scarce concerning *the chronic forms of hepatitis* mainly because these diseases are poorly classified and relatively uncommon These cases are characterized by a protracted course sometimes lasting for years with biochemical evidence of con tinued activity and a histological picture of chronic inflammation without cirrhosis Periodic exacerba tions are common and the spontaneous prognosis is far more grave than that of acute hepatitis A num ber of these patients show evidence of auto-im munization with associated manifestations from other organs such as the joints and serous mem *branes and they may have positive L E cell tests* and raised serum gamma globulins For these rea sons most authorities use steroids in the treatment of chronic hepatitis The results are difficult to evaluate because one cannot compare the treated and untreated groups of patients usually steroids are given for ethical reasons sooner or later if the course is unfavourable Typical in this respect is Schmid s material (12) in which 54 patients were observed for up to 6 years with biopsy control 44 of these patients including those who were most ill received continuous or intermittent steroid therapy whilst only 10 served as untreated controls Of these last 7 recovered or improved as compared with 19 out of 44 in the steroid group This leads to the conclusion which is also supported by other reports (1, 3 8 9 10) that steroids do not have any decisive influence on the outcome and histological picture (as seen in repeated biopsies) in chronic *hepatitis This is also true of patients with evidence* of auto immunization On the other hand it is often possible to achieve a superficial and transient im provement in the general condition subjective feeling of well being and a few of the biochemical para meters particularly the serum bilirubin level

It is difficult to make any general remarks about the toxic or drug induced liver diseases This is partly because these disorders differ so much in their pathogenesis and thereby in the possible effect of the steroids and partly because there are no con trolled therapeutic trials The spontaneous course is often satisfactory if it is possible to remove the offending causative agent and steroid therapy should be contemplated only in cases with either a fulminant or an exceptionally persistent course The types which may be expected to respond best are of course the drug induced liver disorders of allergic origin characterised by either liver cell necrosis or *intrahepatic cholestasis but even here the results are* not convincing, and they are in no way comparable with those obtained with steroid treatment of the true allergic diseases

The last group is *cirrhosis* the frequency and grave spontaneous prognosis of which make it espe cially desirable to find an effective therapy Despite the fact that the steroids have been used in the treat ment of cirrhosis for almost 20 years it has only recently become possible to found an evaluation of their effect on a solid statistical basis The reasons for this are those usual in therapeutic investigations the early materials were too small there were either no control groups or those reported were not com parable the diagnostic criteria were not uniform and often without bioptic verification the periods of observation were too short and insufficient allow ance was made for the varied aetiology of cirrhosis and for its unpredictable individual spontaneous course In general the conclusions reached were encouraging Harvald & Madsen (5) compared 66 patients with generally non alcoholic cirrhosis who were treated with prednisone with 89 patients who had not received steroids However the satisfactory results of the treatment must as the authors them selves point out be accepted with the reservation that the two groups were from different periods

The question of the value of steroid therapy in cirrhosis still needed a final answer which could only be gained from an investigation which satisfied the following basic requirements a uniform diagnosis verified by biopsy and a sufficiently large material of patients observed for a long period in which the effect of the treatment could be judged in comparison with an adequate contemporary control group This was our aim when on January 1st 1962 a coordinated study was started in 7 medical departments in Copenhagen It would take too long to report here full details of the method and preliminary results of this large investigation but a few of the main lines and illustrative examples will be given

It was originally intended to include all patients who fulfilled the histological criteria of cirrhosis of which the most important is the proliferation of connective tissue with disappearance of the normal architecture of the liver lobules However it proved necessary to exclude about half of such patients either because they had previously received steroids or because it was impossible to obtain the necessary cooperation To date the material comprises 525 patients who are divided into treatment and control groups according to their date of birth Prednisone and placebo are administered in doses according to the individual course initially always 40 mg and never less than 10 mg daily as maintenance dosage The remaining treatment of the cirrhosis and its complications has been standardized and identical in both groups with the exception that in critical situations the placebo patients have not received steroids by injection On admission to the material at death in cases of deterioration or of complications and otherwise at regular intervals the patients case history clinical biochemical and where possible histological data have been registered on detailed forms and the material processed for electronic data evaluation

In its composition the material closely corresponds to those usual in Scandinavia there are 40 % women and 40 % who have a history of alcoholism The steroid and placebo groups have proved to be comparable in all important respects with regard to clinical biochemical and histological findings

The patients treated with prednisone achieved a certain statistically significant degree of improvement but only during the first year of the observation period thereafter there was no difference between the two groups This indisputable but transient improvement was noticed in fatigue anorexia hepatomegaly and raised levels of serum bilirubin GO transaminase and gamma globulin

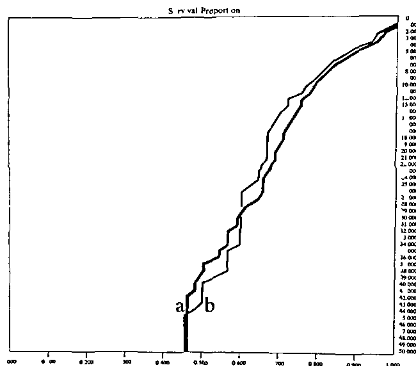


Fig 1 Survival graphs in patients with cirrhosis treated with prednisone (b) and placebo (a)

With regard to the crucial question whether the steroids have any effect on the mortality there is however no difference between the groups whose survival rates are shown in fig 1 The figure which shows the way in which the data processing machine delivers its answers also provides a reminder that cirrhosis of the liver is a serious disease with a 50% mortality at 3½ years Before accepting the obvious conclusion that steroids have no effect on the survival in cirrhosis we have considered it necessary to test two other possibilities The first is that prednisone might have a beneficial effect on one category of patients and an equal but negative effect on another At the present stage of the investigation it is difficult to exclude this possibility there seem to be a tendency for female patients without ascites to benefit from steroids and conversely for steroid to be harmful to patients with ascites on the other hand we have found that the survival time is not affected by any such negative effects (shock infections surgical complications etc) as might be ascribed to steroid no significant difference has been found between the causes of death in the two groups Incidentally the undesirable actions of the steroids were less disturbing than we had expected this is discussed in more detail elsewhere in the symposium

The second possibility which is equally difficult to test is that the steroids might in fact have had a beneficial effect on survival but in a category of patients so small that the effect is hidden by the observation uncertainty In an attempt to find such patients who might have benefited from steroids the material has been divided into small groups according to numerous different criteria As an example fig 2 shows the survival graphs for the treated and control groups when attention is paid to whether or not the patient had palmar erythema on admission to the trial It is clearly apparent that this symptom is correlated to a poor prognosis, but that otherwise there is no difference between the survival graphs The same is found in fig 3 where the patients are divided according to whether the prothrombin value was above or below 60% on admission to the material

A total of about 60 different survival graphs of this type have been constructed without any significant differences having been found

Combinations of the different criteria have also been tested Of these the constellation of female sex no alcoholism no ascites and obvious hypergammaglobulinaemia was of special interest The figures in this group would suggest that these patients have a better chance of survival when treated with steroids

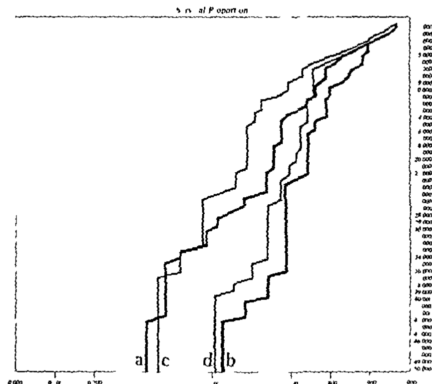


Fig 2 Survival graphs in patients with cirrhosis treated with prednisone (a and b) and placebo (c and d) The two graphs on the left represent patients who on admission to the material had palmar erythema those on the right represent patients without palmar erythema

than when not so treated but the difference is not statistically significant and it is still too early to determine whether it will disappear or be confirmed with time

If in conclusion one tries to give a summarizing evaluation of the use of corticosteroids in the treatment of liver diseases this must be that steroids cannot deserve any central position in the treatment of these conditions as their action in liver disease is inconstant in addition to being symptomatic in nature moderate in degree and of limited duration It may be added that it is justifiable to make use of the steroid effect as a symptomatic therapy in liver d s eases with an unfavourable course

As regards cirrhosis of the liver it would seem that the fate of certain limited groups of patients is influenced by steroids but it is too early to verify this statistically

P s After this manuscript was in print renewed statistical analysis confirmed that steroid treatment is associated with a signficant reduction in death rate in female patients without ascites On the other hand, if ascites and alcoholism were present steroid was correlated to an increased death rate irrespective of sex (14)

DISCUSSION

Dr H ... early ... either ... Pres ...

D ... roids ... e of th ...

Dr Q ... steroid on the subjective complaints such as anorexia

D ... In your material of patients with cirrhosis, ha any attempt been mad to evaluate parameters other than the liver function tests and the survival time? It is conceiv able that the pati nts feel better during steroid therapy even if they live no longer

Dr I ersen I would like to put the same question The mor tality rate is the same in both groups, and the incidence of side-effects in the t eated group is not really frightening. It is very apparent when one sees these patients that they feel much better during steroid therapy than when they receive

a
b
c
d

Fig. 3 Survival graphs in patients with cirrhosis treated with prednisone (b c) and placebo (a d) The two graphs on the left represent the patients who on admission to the material had prothrombin proconvertin values of less than 60 / and those on the right the patients in whom the values were above 60 /

placebo The problem here is therefore the same as that in patients with rheumatic arthritis Under which circumstances does the patient feel best, and under which circumstances has he the greatest working capacity?

With regard to acute hepatitis I am in full agreement with Quaade s conclusion The group which is particularly susceptible the postmenopausal women in whom the illness tends to be of long duration should probably in some cases be treated with steroids but this treatment should be absolutely discouraged in the large group of patients with banal hepatitis

In a yet unpublished very large controlled study from Switzerland (Haemmerli) it was found that the mortality rate in acute hepatitis was more than doubled in patients treated with steroids and that the recurrence rate was twice to three times as high

Dr Quaade Those cirrhosis patients who receive steroids feel better during the first year but after this there is no difference We have carefully registered subjective symptoms and also working capacity and the number of weeks the patients stay in bed There is no doubt that in these respects an improvement is obtained during the first year

REFERENCES

1 Ammann R. & Schmid M Die Therapie der chronischen Hepatitis Schweiz. med Wschr 94 498-504 1964

2. Davis, M A., Peters R. L. Redeker A G & Reynolds T B Appraisal of the mortality in acute fulminant viral hepatitis New Eng J Med 278 1248-1253 1968

3 Franken, F H Daweke H Gries E A v Schweinitz H A Holzgrewe H & Forstmann U Langzeitbehandlung chronischer Lebererkrankungen mit Prednison und Methandrostenolon Dtsch med Wschr 41 1979-1985 1963

4 Haemmerli U P Zur Frage der Steroidtherapie der akuten Hepatitis epidemica Schweiz med Wschr 94 504-507 1964

5 Harvald B & Madsen S Long term treatment of cirrhosis of the liver with prednisone Acta med scand 169 381-387 1961

6 Iber F L & Mendeloff A L Prevention and treatment of viral hepatitis Arch intern Med 109 310-3?2 1962

7 Katz, R Velasco M Klinger J & Alessandri H Corticosteroids in the treatment of acute hepatitis in coma Gastroenterology 42 258-265 1962

8 Mackay I R & Wood I J The course and treatment of lupoid hepatitis Gastroenterology 45 4-13 1963

9 Mistilis S P & Schiff L Steroid therapy in chronic hepatitis Arch intern Med 113 104-112 1964

10 Read A E Sherlock S & Harrison C V Active juvenile cirrhosis considered as part of a systemic disease and the effect of corticosteroid therapy Gut 4 378-393 1963

11 Ritis F de Giusti G Mallucci L & Piazza M Negative results of prednisone therapy in viral hepatitis Lancet I 533-534 1964

12 Schmid M Die chronische Hepatitis Experimentelle Medizin Pathologie und Klinik Springer Verlag Band 18 1966

13 Trey C Treatment of fulminant hepatic failure New Eng J Med 278 1287-1288 1968

14 The Copenhagen Study Group for Liver Diseases The effect of prednisone on the survival of patients with cirrhosis of the liver A controlled study Lancet 2 119 121 1968

Acta med scand Suppl 500 pp 55-60 1969

GLUCOCORTICOIDS IN GASTRO ENTEROLOGY

by
Povl Riis

No mention will be made of the prophylactic admin istration of glucocorticoids to avoid late stenosis of the oesophagus or stomach following the ingestion of corrosives nor of the possible ulcerogenic action of these hormones Instead only the direct therapeutic applications of the glucocorticoids in gastro enterology will be described their role in the treatment of liver diseases is dealt with in another paper in the symposium There remains for discussion the therapeutical value of glucocorticoids in non tropical sprue (coeliac disease) Crohn s regional enteritis and ulcerative colitis

The evaluation of the effect of glucocorticoid therapy in these disorders has the common feature that the effect must be compared with two or three alternative modes of treatment and cannot be considered *in vacuo*

NON TROPICAL SPRUE
COELIAC DISEASE

The first line of treatment of the adult form is always a gluten free diet whilst glucocorticoids take second place An important clinical question is in what proportion of patients dietetic therapy alone is sufficient to produce remission how often supplementary glucocorticoid therapy is necessary and how often both therapeutic regimens fail The disorder is so rare that very few gastro enterological departments see more than a few cases annually which means that the majority of gastro enterologists only have clinical experience of single cases and no opportunity to systematize and check this experience on a larger scale Two recent studies have supplemented our present knowledge (5 19)

In one of these from England (19) 54 patients are reported The diagnosis was made solely on the presence of a flat jejunal mucosa The observation time varied from six months to 14 years Gluten free diet led to remission in 70 % whilst the remaining 30 % showed all degrees of therapeutic failure These last cases were divided into three groups 1) Five patients (who had the characteristic of showing an almost complete lack of Paneth cells) were treated with supplementary prednisone (about 60 mg daily initially falling to 10–20 mg daily) In three of these patients the combined therapy arrested the illness whilst in two it was unsuccessful 2) Three patients who also suffered from pancreatic failure did not respond to diet plus prednisone 3) Three of the remaining eight in this group were suspected of repeated minor deviations from the dietetic regimen and they gradually recovered None of this group received glucocorticoids

In the second study from U S A (5) 22 patients were followed up after an observation period of one to ten years The diagnosis was established on the basis of the clinical history objective examination absorption tests X ray findings and biopsies of the small intestine Seventeen of the 22 patients remitted on a gluten free diet whilst five required supplementary glucocorticoids after a six month trial of diet The group which required glucocorticoids had poorer results in absorption tests than the group who remitted on diet alone this was true both initially and at follow up

A preliminary report of the use of betamethasone 17 valerate without simultaneous diet has recently been published (18)

The collective conclusion of the two first studies is that approx one quarter of patients with non tropical sprue do not remit on dietetic therapy alone and that a supplementary treatment with glucocorticoids may be expected to lead to remission in many of these patients although probably not in all The discrepancy between the two studies on this point is presumably a result of the different diagnostic criteria which have been applied It is hardly reasonable to wait for more than six months before starting glucocorticoid therapy in patients who do not show

a satisfactory response to diet On the other hand the observation period for the dietetic therapy should not be too short as repeated deviations from the dietetic regimen must be accepted as almost inevitable during the first two or three months

REGIONAL ENTERITIS CROHN S DISEASE OF THE SMALL INTESTINE

With the exception of the treatment of malignant tumours, the treatment of Crohn s disease constitutes one of the most serious therapeutical problems in gastro-enterology As in other branches of clinical medicine there seems to be an inverse proportion between the number of therapeutic approaches suggested and the effect of treatment Little (if anything) apart from glucocorticoids and operation has any demonstrable effect on the course of the illness if a necessary substitution therapy is considered as secondary Many patients with mild Crohn s disease of the small intestine manage without either medical or surgical treatment It is not until a certain threshold in the intensity of the illness is exceeded usually pronounced diarrhoea and severe abdominal pain with reduction in the general condition of the patient that it is necessary to make any decision with regard to glucocorticoid therapy On the other hand this treatment should always be tried before resorting to operation unless the patient is seen for the first time at such a late stage that the two complications indicative of operation are already present severe stenosis and/or severe functionally short-circuiting, fistulation

Reference is made to two investigations as a supplement to personal experience and as keys to the literature on the subject Neither of these was carried out as a controlled study

Sparberg & Kirsner (24) in Chicago, selected retrospectively a group of 54 patients (32 males 22 females) who had received at least six months of continuous glucocorticoid therapy usually with prednisone and carried out a follow up after various times which are not stated in detail The primary results were considered excellent in 45 cases and good in 11 whilst the late results were excellent in 19 and good in ten Operation had proved necessary in 19 cases The authors concluded that it is possible to achieve a symptom free state with glucocorticoids in about 50 % of patients and that the results are best when treatment is given early in the course and when the patients are under the age of 45 years The investigation can be criticized on the following grounds 1) Those patients on whom operations were performed before the conclusion of six months of glucocorticoid therapy must be assumed to have been severely affected and the distribution of the group studied is therefore skew 2) It was not possible to correct for the spontaneous fluctuating course of the illness, and the postulated causality between the treatment and the course observed is therefore highly disputable

A more conclusive retrospective study is that reported by Jones et al (8) from the Central Middlesex Hospital in London of 105 patients who attended between 1954 and 1964 Thirty of these patients were treated with corticotrophin or glucocorticoids Crohn s disease of both small and large intestine and combined forms were represented Among the encouraging primary results were three patients who went into complete remission and a further 19 who showed improvement On follow up the remission had been maintained in only one patient whilst four out of the 22 had died and eight had undergone operation Seven of the patients in whom operation was contra indicated were maintained in partial remission on long term glucocorticoid therapy No particular group of patients reacted more satisfactorily than any other The main indications for glucocorticoid therapy were stated to be wide spread involvement of the small intestine and recurrence after a previous resection

ULCERATIVE COLITIS

Any discussion of the treatment of this disease necessitates a brief description of its diagnostic definition and its delimitation from Crohn s disease of the colon which may or may not be a different disease entity and which according to the former theory should be treated in a manner different from ulcerative colitis

Our diagnostic definition of ulcerative colitis is as follows (21)

The diagnosis ulcerative colitis which comprises all stages from haemorrhagic proctitis to total ulcerative colitis is based on the presence of two of the following four symptoms or signs 1) A clinical history with report of macroscopic blood on the faeces or passage of blood between defaecations Diarrhoea and/or rectal urgency not obligatory 2) Typical findings on sigmoidoscopy (at least two

Table I Treatment of ulcerative colitis

		COURSE		
		ACUTE	CHRONIC INTERMITTENT	CHRONIC CONTINUOUS
	RECTUM	C (I)	SP (L) 2 g daily Possibly C (I) (S)	SP (L) 2 g daily Not C (I) Not C (G)
EXTENT	RECTUM + SIGM			
	RECTUM + SIGM + DESC	C (G) (I) (S) Possibly SP (S) 4–6 g daily	SP (L) 2 g daily Possibly C (G) (I) (S)	SP (L) 2 g daily Rarely C (G) (L)
	RECTUM + SIGM + DESC + TRANS		If several failures OPERATION	Often OPERATION
	WHOLE COLON	C (G) (S) for 4–6 days daily surgical medical observation *Possibly* combined with SP (S) 4–6 (–8) g daily If no dramatic remission OPERATION	SP (L) 2–3 g daily Possibly C (G) (I) (S) If several failures *OPERATION*	Usually OPERATION

C = glucocorticoid therapy
SP = salazo-sulphapyridine therapy
(L) = long term therapy
(S) = short term therapy
(G) = systemic therapy
(I) = instillation therapy

present) a) granulation b) friability c) haemorrhagic purulent contents of lumen d) small or large ulcers and e) pseudopolyps 3) Typical cytological and/or biopsy findings a) inflammatory leucocytes in cytological preparations (eosinophilic granulocytes not obligatory) b) inflammatory leucocytes in mucosa and submucosa of histological preparations 4) Typical radiological findings a) spicula (not deep fissures) b) small (less than 2 mm) or undermining ulcers and c) pseudopolyps

(Fistulae stenosis rigidity and loss of haustration are considered non specific In patients with diverticula the requirements are increased to three instead of two of the cardinal symptoms and signs which in the present paper have been given arabic numbers)

It should be mentioned that Crohn s disease of the colon as delimited by our criteria (21) has not been included in the following review of ulcerative colitis but will be mentioned in brief at the end of the paper

Before giving a review of the position of glucocorticoid therapy in the treatment of ulcerative colitis it is necessary to mention two important classifications of these cases One is according to anatomical extent and the second to the type of course whether acute chronically intermittent or chronically continuous We require an observation period of two years before a patient is classified according to the latter system Table I contains a review of glucocorticoid salazo sulphapyridine and operative treatment in cases with different degrees of bowel involvement and various types of course The contents of the table are based on a few controlled investigations and in addition on our personal experience with up to the present 330 ulcerative colitis patients the majority of whom have regularly attended out patient follow up

With a minor reservation for the limitations of a schematic presentation this review should provide adequate support in the choice of present day therapy in any given patient with ulcerative colitis For reasons of space supplementary comments on the individual parts of the table will be omitted with two exceptions These involve the first and second columns third row where it is stated that operation may be necessary if antecedent glucocorticoid therapy has proved insufficient The question arises as

a satisfactory response to diet On the other hand the observation period for the dietetic therapy should not be too short as repeated deviations from the dietetic regimen must be accepted as almost inevitable during the first two or three months

REGIONAL ENTERITIS CROHN S DISEASE OF THE SMALL INTESTINE

With the exception of the treatment of malignant tumours the treatment of Crohn s disease constitutes one of the most serious therapeutical problems in gastro-enterology As in other branches of clinical medicine there seems to be an inverse proportion between the number of therapeutic approaches suggested and the effect of treatment Little (if anything) apart from glucocorticoids and operation has any demonstrable effect on the course of the illness if a necessary substitution therapy is considered as secondary Many patients with mild Crohn s disease of the small intestine manage without either medical or surgical treatment It is not until a certain threshold in the intensity of the illness is exceeded usually pronounced diarrhoea and severe abdominal pain with reduction in the general condition of the patient that it is necessary to make any decision with regard to glucocorticoid therapy On the other hand this treatment should always be tried before resorting to operation unless the patient is seen for the first time at such a late stage that the two complications indicative of operation are already present severe stenosis and/or severe functionally short-circuiting fistulation

Reference is made to two investigations as a supplement to personal experience and as keys to the literature on the subject Neither of these was carried out as a controlled study

Sparberg & Kirsner (24) in Chicago selected retrospectively a group of 54 patients (32 males 22 females) who had received at least six months of continuous glucocorticoid therapy usually with prednisone and carried out a follow up after various times which are not stated in detail The primary results were considered excellent in 45 cases and good in 11 whilst the late results were excellent in 19 and good in ten Operation had proved necessary in 19 cases The authors concluded that it is possible to achieve a symptom free state with glucocorticoids in about 50 % of patients and that the results are best when treatment is given early in the course and when the patients are under the age of 45 years The investigation can be criticized on the following grounds 1) Those patients on whom operations were performed before the conclusion of six months of glucocorticoid therapy must be assumed to have been severely affected and the distribution of the group studied is therefore skew 2) It was not possible to correct for the spontaneous fluctuating course of the illness and the postulated causality between the treatment and the course observed is therefore highly disputable

A more conclusive retrospective study is that reported by Jones et al (8) from the Central Middlesex Hospital in London of 105 patients who attended between 1954 and 1964 Thirty of these patients were treated with corticotrophin or glucocorticoids Crohn s disease of both small and large intestine and combined forms were represented Among the encouraging primary results were three patients who went into complete remission and a further 19 who showed improvement On follow up the remission had been maintained in only one patient whilst four out of the 22 had died and eight had undergone operation Seven of the patients in whom operation was contra indicated were maintained in partial remission on long term glucocorticoid therapy No particular group of patients reacted more satisfactorily than any other The main indications for glucocorticoid therapy were stated to be wide spread involvement of the small intestine and recurrence after a previous resection

ULCERATIVE COLITIS

Any discussion of the treatment of this disease necessitates a brief description of its diagnostic definition and its delimitation from Crohn s disease of the colon which may or may not be a different disease entity and which according to the former theory should be treated in a manner different from ulcerative colitis

Our diagnostic definition of ulcerative colitis is as follows (21)

The diagnosis ulcerative colitis which comprises all stages from haemorrhagic proctitis to total ulcerative colitis is based on the presence of two of the following four symptoms or signs 1) A clinical history with report of macroscopic blood on the faeces or passage of blood between defaecations Diarrhoea and/or rectal urgency not obligatory 2) Typical findings on sigmoidoscopy (at least two

choice between medical and surgical treatment is very difficult (see table I) No clinical characteristics can distinguish the group of patients in whom there will be a satisfactory remission with glucocorticoid (or a combination of glucocorticoid and salazo sulphapyridine) therapy An attempt to correlate satisfactory response to glucocorticoid therapy with the presence of eosinophilic granulocytes in the colo nic inflammatory exudate before treatment was com menced has been made in our department by Mar cussen & Ærenlund Jensen The results were nega tive (12)

Glucocorticoids may be indicated in cases of severe ulcerative colitis in pregnant women (35) The risk of foetal damage seems to be negligible In contrast to this is a recent report of a relatively high incidence of foetal death in utero in women who received long term glucocorticoid therapy during pregnancy (33)

In order to determine whether partial adrenal hypofunction is ever present in untreated ulcerative colitis patients Binder & Binder have carried out tests of adrenal function in about 50 patients from our department Their preliminary results are nega tive

The controversial clinical picture *Crohn s disease of the colon* has as yet not been the subject of any controlled trial of glucocorticoid therapy In a review from January 1968 (10) it is stated that sulphonamides or corticosteroids sometimes relieve the acute symptoms but surgical treatment is often necessary later The uncertain definition of this condition which has been mentioned above makes it impossible at the moment to give any critical evaluation of the various forms of treatment

DISCUSSION

Dr Kirketerp I would recommend a considerably higher dosage of glucocorticoids in acute ulcerative colitis involving the entire colon up to about 200 mg prednisone daily We have observed dramatic improvement in five patients in whom we have used this dosage

Dr Loren en Is it not a general experience with the gluco corticoids that after the administration of very high doses it is mainly a rapid effect which is achieved whilst in the long run the use of such doses is no more effective than the administration of low doses and the incidence of side-effects is much lower in the latter

Dr Worning With reference to the question of the increased risk of surgery in those patients with ulcerative colitis who are receiving treatment with steroids can the administration of very high doses of glucocorticoids which are often given as a routine in connection with operation be of any im portance in the complication rate?

In Rigshospitalet we have used a pre-operative investiga tion of the pituitary adrenal cortical function in these patients as a guide to whether or not supplements of glucocorticoids should be given in connection with operation It would appear that as a rule it is superfluous to administer very high doses and that the avoidance of these has reduced the in cidence of complications of surgery

A second question When it is found that there are in dications for the use of glucocorticoids is there any differ ence in the risks of using, for example 60 mg prednisone and those of using four times that dose as long as the plans concerning the duration of the treatment are completely fixed?

Dr Sprechler Where the operative risks in these patients are concerned I do not believe that it is of any importance whether or not they have received glucocorticoids The in creased operative risks are found firstly in those in whom operation has been postponed for too long and whose gen eral condition is therefore poor The second group com prises the patients with primary and rapidly developing toxic megacolon These patients should not be treated medically but should be submitted to operation immediately

Dr Riis We are afraid of masking perforation if we apply too high a dose of steroids but I can give no documentation from controlled materials on the difference between the effect of various doses I do not consider that the natural course of ulcerative colitis is affected by any treatment other than sur gery Patients who have suffered from pancolitis on one occasion always as far as we know d v lop a recurrence sooner or later The same group of patient h ve an increased risk of cancer after a number of years and therefore we do not regret having to operate on a few extra patients who might have been brought out of the actual exacerbation by the use of more heroic doses of glucocorticoids

With regard to the question of increased supplements of glucocorticoids in connection with colectomy during steroid therapy we have followed the same guiding lines as Worning Al o we doubt the value of routine administration On the other hand I do not know whether any pre-operative glucocorticoid treatment increases the risk of surgery I com pletely agree with Sprechler that the most important cause of a high risk in some ulcerative colitis patients is that operation is not considered until too late

REFERENCES

1 Baron J H Connell A M Kanaghinis F G Len nard Jones J E & Jones F A Out patient treatment of ulcerative colitis Comparison between three doses of oral prednisone Brit med J II 441-443 1962
2 Binder Vibeke & Binder Chr Unpublished results
3 Connell A M., Lennard Jones J E Misiewicz J J Baron J H & Jones F A Comparison of acetarsol and prednisolone 21 phosphate suppositories in the treatment of idiopathic proctitis Lancet I 238 239 1965
4 Cocco A E & Mendeloff A I An evaluation of inter mittent corticosteroid therapy in the management of

to whether such antecedent glucocorticoid therapy might not increase the operative and post-operative mortality It is not possible to refer to any controlled investigations which give an unequivocal answer to this question It is undoubtedly true that the impression of a large number of operative complications in patients receiving long term glucocorticoid therapy which has been reported by surgeons interested in ulcerative colitis is based on reality but it is improbable that the cause is merely the glucocorticoid therapy It is more likely that operation late in the course of an acute severe ulcerative colitis is always associated with an increased mortality rate regardless of which inadequate medical treatment has led to the postponement of the decision to operate This is the basis for the decision to give only 4–6 (–8) days of glucocorticoid therapy in the fulminating case unless there is a *dramatic* change in the patient s condition within that period

A short account will be given of the most important investigations into the effect of the glucocorticoids in ulcerative colitis

In 1955 Truelove & Witts introduced the use of cortisone in a controlled trial (27) Of a group of 210 patients 109 received 100 mg cortisone daily and the remainder placebo There was a significant effect of the treatment which was most commonly observed in patients suffering from their first attack In 1960 Truelove (31) reported three sets of 40 courses of treatment in 105 patients one group received 5 mg prednisone four times daily one local treatment with either hemisuccinate or prednisolone 21 phosphate and one group a combination of these forms of treatment The results were best in group three remission being induced in over three quarters of the group

Baron et al (1) titrated the dosage of prednisone in a controlled trial and demonstrated that 40 mg daily was preferable to 20 mg or 60 mg, and that the full effect was obvious within a fortnight

An attempt has been made to compare the effect of 20–60 mg prednisone daily given in divided doses with that of 40–120 mg on alternate days (4) The conclusion that the adrenal suppression was less during the intermittent treatment is presumably justified whilst in contrast the documentation of the identical clinical effect in the two groups is unconvincing

At the present moment it would not seem that betamethasone 17 valerate exhibits any advantages in the treatment of ulcerative colitis

Local therapy by rectal instillation was introduced by Truelove in 1956 (28) and has since been shown to be effective in several investigations (11 15 23 26 29, 34) although to date it has not been proved that the effect is due to a primary direct action on the mucous membrane Matts & Gaskell (13) have demonstrated that a fluid instilled per rectum can carry an agent high up into the colon especially in ulcerative colitis These workers used an enema technique with plastic containers in contrast to the rectal drip instillations which had been used previously There is documentation for the fact that 20 %–80 % of cortisol-esters (least for prednisone) administered rectally is absorbed and that the absorbed drug affects the endogenous production of cortical hormone in the expected manner (16 17 22 25, 36)

Truelove has demonstrated the probability of a beneficial effect of a combination of systemic and local therapy (31), and on the basis of a small uncontrolled series Matts (14) has recommended the combination in severe cases Recently the technique of rectal instillation has been modified by workers at St Mark s Hospital and the high costs associated with this form of administration have thereby been reduced (7)

To summarize with regard to local treatment with glucocorticoids it may be stated that in many cases this form of administration does not have any convincing advantages as compared with systemic treatment with prednisone especially as a large part of the effect is undoubtedly achieved via absorption Now and then the beneficial effect of this form of administration in a patient in whom the disease is localized distally justifies its disadvantages and higher cost

Truelove et al (32) have made a direct comparison between 20 mg prednisone plus 100 mg hydrocortisone hemisuccinate daily and 8 g salazo sulphapyridine daily using a sequential analysis Glucocorticoids proved to be the more effective in the treatment of the acute case

A variant of local treatment is the use of glucocorticoid suppositories which in some investigations have been found to be beneficial in mild cases with a distally localized disease (3 9 30) Our own experience from a double blind study is that this form of treatment does not deserve a place in the therapeutic armamentarium (20)

When faced with an acute case of ulcerative colitis involving the whole – or most – of the colon the

choice between medical and surgical treatment is very difficult (see table I) No clinical characteristics can distinguish the group of patients in whom there will be a satisfactory remission with glucocorticoid (or a combination of glucocorticoid and salazo sulphapyridine) therapy An attempt to correlate satisfactory response to glucocorticoid therapy with the presence of eosinophilic granulocytes in the colonic inflammatory exudate before treatment was commenced has been made in our department by Marcussen & Ærenlund Jensen The results were negative (12)

Glucocorticoids may be indicated in cases of severe ulcerative colitis in pregnant women (35) The risk of foetal damage seems to be negligible In contrast to this is a recent report of a relatively high incidence of foetal death in utero in women who received long term glucocorticoid therapy during pregnancy (33)

In order to determine whether partial adrenal hypofunction is ever present in untreated ulcerative colitis patients Binder & Binder have carried out tests of adrenal function in about 50 patients from our department Their preliminary results are negative

The controversial clinical picture *Crohn s disease of the colon* has as yet not been the subject of any controlled trial of glucocorticoid therapy In a review from January 1968 (10) it is stated that sulphonamides or corticosteroids sometimes relieve the acute symptoms but surgical treatment is often necessary later The uncertain definition of this condition which has been mentioned above makes it impossible at the moment to give any critical evaluation of the various forms of treatment

DISCUSSION

Dr Kirketerp I would recommend a considerably higher dosage of glucocorticoids in acute ulcerative colitis involving the entire colon up to about 200 mg prednisone daily We have observed dramatic improvement in five patients in whom we have used this dosage

Dr Loren en Is it not a general experience with the glucocorticoids that after the administration of very high doses it is mainly a rapid effect which is achieved whilst in the long run the use of such doses is no more effective than the administration of low doses and the incidence of side-effects is much lower in the latter?

Dr Worning With reference to the question of the increased risk of surgery in those patients with ulcerative colitis who are receiving treatment with steroids can the administration of very high doses of glucocorticoids which are often given as a routine in connection with operation be of any importance in the complication rate?

In Rigshospitalet we have used a pre operative investigation of the pituitary adrenal cortical function in these patients as a guide to whether or not supplements of glucocorticoids should be given in connection with operation It would appear that as a rule it is superfluous to administer very high doses and that the avoidance of these has reduced the incidence of complications of surgery

A second question When it is found that there are indications for the use of glucocorticoids is there any difference in the risks of using for example 60 mg prednisone and those of using four times that dose as long as the plans concerning the duration of the treatment are completely fixed?

Dr Sprechler Where the operative risks in these patients are concerned I do not believe that it is of any importance whether or not they have received glucocorticoids The increased operative risks are found firstly in those in whom operation has been postponed for too long and whose general condition is therefore poor The second group comprises the patients with primary and rapidly developing toxic megacolon These patients should not be treated medically but should be submitted to operation immediately

Dr Riis We are afraid of masking perforation if we apply too high a dose of steroids but I can give no documentation from controlled materials on the difference between the effect of various doses I do not consider that the natural course of ulcerative colitis is affected by any treatment other than surgery Patients who have suffered from pancolitis on one occasion always as far as we know d clop a recurrence sooner or later The same group of patient have an increased risk of cancer after a number of years and therefore we do not regret having to operate on a few extra patients who might have been brought out of the actual exacerbation by the use of more heroic doses of glucocorticoids

With regard to the question of increased supplements of glucocorticoids in connection with colectomy during steroid therapy we have followed the same guiding lines as Worning Also we doubt the value of routine administration On the other hand I do not know whether any pre-operative glucocorticoid treatment increases the risk of surgery I completely agree with Sprechler that the most important cause of a high risk in some ulcerative colitis patients is that operation is not considered until too late

REFERENCES

1 Baron J H Connell A M Kanaghinis F G Lennard Jones J E & Jones F A Out patient treatment of ulcerative colitis Comparison between three doses of oral prednisone Brit med J II 441–443 1962
2 Binder Vibeke & Binder Chr Unpublished results
3 Connell A M Lennard Jones J E Misiewicz J J Baron J H & Jones F A Comparison of acetarsol and prednisolone 21 phosphate suppositories in the treatment of idiopathic proctitis Lancet I 238–239 1965
4 Cocco A E & Mendeloff A I An evaluation of intermittent corticosteroid therapy in the management of

ulcerative colitis Johns Hopkins med J 120 162 169 1967
5 Finkel M Gelb A M Cohen N Ho P P & Janowitz, H D Long term follow up study in idiopathic steatorrhoea Amer J Gastroent 47 35–40 1967
6 Friedman M Fletcher J Hinton J M Lennard Jones J E Misiewicz, J J & Parrish J A Observations on the absorption of oral betamethasone 17 valerate and its therapeutic value in ulcerative colitis Brit med J I 335–337 1967
7 Jones J H Lennard Jones J E & Misiewicz, J J Modified technique for administration of retention enemata in colitis Gut 6 514 1965
8 Jones J H & Lennard Jones J E Corticosteroids and corticotrophin in the treatment of Crohn s disease Gut 7 181–187 1966
9 Lennard Jones J E Baron J H Connell A M & Jones F A A double blind controlled trial of prednisolone 21 phosphate suppositories in the treatment of idiopathic proctitis Gut 3 207–210 1962
10 Lennard Jones J E Medical aspects of Crohn s disease Proc roy Soc Med 61 81–83 1968
11 MacDougall I Treatment of ulcerative colitis with rectal steroids Lancet I 826–827 1963
12 Marcussen H & Ærenlund Jensen H Den prognostiske betydning af inflammatorisk eosinofili for effekten af kortikosteroidbehandling ved colitis ulcerosa Ugeskr Læg 130 129–133 1968
13 Matts S G F & Gaskell K H Retrograde colonic spread of enemata in ulcerative colitis Brit med J II 614–616 1961
14 Matts S G F Combined steroid therapy of fulminating ulcerative colitis Brit med J I 1045–1048 1962
15 Matts S G F Betamethasone enemata in ulcerative colitis Gut 3 312–314 1962
16 Matts S G F Wharton B A Kelleher J & Walters G Adrenal cortical and pituitary function after intra rectal steroid therapy Brit med J II 24–26 1963
17 Nabarro J D N Moxham Audrey Walker G & Slater J D H Rectal hydrocortisone Brit med J II 272 274 1957
18 Otaki A T Daly J R & Morton Gill A Observations on oral betamethasone 17 valerate in the treatment of idiopathic steatorrhoea Gut 8 458–462 1967
19 Pink, I J & Creamer B Response to a gluten free diet of patients with the cœliac syndrome Lancet I 300–304 1967
20 Riis, P Søgaard Andersen T & Anthonisen P Behandling med fenylbutazon og prednisolonsuppositorier ved ikke specifik hæmoragisk proctocolitis Ugeskr Læg 126 792–796 1964
21 Riis P & Anthonisen P 8th International Congress of Gastroenterology Prague July 1968
22 Sampson P A & Brooke B N Absorption of hydrocortisone from the large bowel Lancet I 701–702 1963
23 Sherman L F & al Treatment of ulcerative colitis with hydrocortisone alcohol retention enemas a topical and systemic anti inflammatory agent Dis Colon Rect 10 43–52 1967 (The author has had no access to this report before publication)
24 Sparberg M & Kirsner J B Long term corticosteroid therapy for regional enteritis an analysis of 58 courses in 54 patients Amer J dig Dis 11 865–880 1966
25 Spencer Jean A Kirsner J B & Palmer W L Rectal absorption of 6-alpha C H_3 prednisolone Proc Soc exp Biol Med 103 74–77 1960
26 Spencer Jean A & Kirsner J B Experience with short and long term courses of local adrenal steroid therapy for ulcerative colitis Gastroenterology 42 669–677 196?
27 Truelove S C & Witts L J Cortisone in ulcerative colitis Brit med J II 1041–1048 1955
28 Truelove S C Treatment of ulcerative colitis with local hydrocortisone Brit med J II 1267–1272 1956
29 — Treatment of ulcerative colitis with local hydrocortisone hemisuccinate sodium Brit med J II 1073–1077 1958
30 — Suppository treatment of haemorrhagic proctitis Brit med J I 955–958 1959
31 — Systemic and local corticosteroid treatment in ulcerative colitis Brit. med J I 464–467 1960
32 Truelove S C Watkinson G & Draper G Comparison of corticosteroid and sulphasalazine therapy in ulcerative colitis Brit med J II 1708–1711 1962
33 Warrell D W & Taylor R Outcome for the fœtus of mothers receiving prednisolone during pregnancy Lancet I 117–118 1968
34 Watkinson G Treatment of ulcerative colitis with topical hydrocortisone hemisuccinate sodium Brit med J II 1077–1082 1958
35 Weeke Eva Binder Vibeke Olsen J H Anthonisen P & Riis P Graviditet og ikke-specifik hæmoragisk proctocolitis (proctitis haemorrhagica og colitis ulcerosa) Nord Med 75 92–96 1966
36 Wood W A Walthers G & Matts S G F Urinary excretion of prednisolone after intrarectal therapy in ulcerative colitis Brit med J II 1045–1046 1964

Acta med scand Suppl 500 pp 61-66 1969

GLUCOCORTICOIDS IN NEPHROLOGY

by
Herluf Jensen

In the field of nephrology it is certain forms of the nephrotic syndrome which form the most important indications for glucocorticoid therapy However mention will first be made of other indications

Attempts to treat post streptococcal diffuse glomerulonephritis with cortisone and ACTH have been unsuccessful (1 16) The same is true of subacute and chronic glomerulonephritis (1 2 16) However the total number of cases of glomerulonephritis which have been treated with glucocorticoids is too small for any definite statement to be made on this question

Glucocorticoids have been used with good results in some cases of focal glomerulonephritis caused by systemic diseases such as systemic lupus erythematosis (SLE) periarteritis nodosa or Schonlein Henoch s disease Only SLE nephropathy will be described in more detail

Since the introduction of renal biopsy it has become apparent that SLE nephropathy includes a wide range of renal disorders from focal glomerulitis through acute and subacute to chronic glomerulonephritis It is important to emphasize that it is possible to find indubitable evidence of SLE in renal biopsies even in cases in which there is no clinical evidence of renal disease and also that during an acute attack of SLE there may for example be proteinuria without histological evidence of SLE nephropathy (15) This is the reason that the incidence of SLE nephropathy in SLE is given variously as from 53 % to 76 % (15) A clinical nephrotic syndrome is found in about 30 % of cases of SLE nephropathy It is particularly younger persons who suffer from SLE nephropathy and it is generally found during the first attack of the illness or put in another way if there is no histological evidence of SLE nephropathy at the first sign of SLE then there is very little risk of this developing later The involvement of the kidneys is generally considered to be a serious complication and although the introduction of renal biopsy has disclosed less severe cases this is still true The different reports of glucocorticoid therapy in SLE nephropathy are based on materials which are often small and difficult to compare among other things because the severity of the nephropathy has varied In the earlier studies in which low dosage of glucocorticoids was used there was no definite effect of the treatment but after the increase in dosage and the duration of treatment a doubling of the survival time has been observed (11) It is important that treatment be started before chronic azotaemia and hypertension develop If this is not the case then because of their catabolic effect the glucocorticoids may lead to a deterioration in the uraemia As mentioned above it is necessary to give high doses for a long time for example ½-1 mg prednisone per kg body weight daily for about six months In consideration of the poor prognosis of SLE nephropathy some workers have recommended that glucocorticoids should never be withdrawn where there is histologically verified involvement of the kidney

As regards the influence of treatment on the histological changes Pollak et al (11) by means of repeated renal biopsies demonstrated that there was no reduction in the lesions in patients receiving small doses of glucocorticoids (50 mg cortisone) but that there was in fact deterioration in the glomerular lesions In patients treated with large doses of prednisone for long periods there was in contrast an improvement apart from the thickening of the basement membrane and synecchiae which persisted Treatment with cytotoxic drugs seemed to be more effective than the glucocorticoids in this respect (7)

In more recent years glucocorticoids have been used as part of the immunosuppressive therapy in renal transplantations Finally for the sake of completeness it may be mentioned that glucocorticoids are used in hypercalcaemic nephropathy due for example to sarcoidosis bone metastases vitamin D

intoxication and myelomatosis and in acute radiation nephritis

In the following glucocorticoid therapy in the nephrotic syndrome will be described in some detail The nephrotic syndrome is characterized by massive proteinuria (> 3 5 g/24 hours at normal glomerular filtration rate) together with varying degrees of dysproteinaemia and hyperlipaemia The nephrotic syndrome can be divided into congenital and acquired and the latter again into primary and secondary The syndrome is called secondary if it is of known aetiology as for example in diabetic intercapillary glomerulosclerosis amyloidosis renal vein thrombosis poisoning and SLE or other connective tissue disease In primary nephrotic syndrome the aetiology has not been clarified but it is thought that immunological factors are responsible Glucocorticoid therapy is specially indicated in cases of primary nephrotic syndome it is occasionally indicated in secondary and without any effect in the congenital nephrotic syndrome

Secondary nephrotic syndromes caused by SLE (see above) periarteritis nodosa and other connective tissue diseases occasionally show a satisfactory response to glucocorticoid therapy whilst on the other hand this is not the case in the nephrotic syndromes caused by diabetes mellitus amyloidosis or renal vein thrombosis

As mentioned above the glucocorticoids are quantitatively of greatest importance in the treatment of primary nephrotic syndromes Before giving a description of the results of therapy certain general problems will be emphasized In the evaluation of the treatment it is important to distinguish between the initial response and the long term prognosis As will be described in more detail later there is a comprehensive literature of the initial response of adult nephrotic syndrome to glucocorticoid therapy whilst on the other hand there are few reports of the long term prognosis

The initial response is usually divided into four types Type A implies complete remission type B the persistence of a slight proteinuria type C that the treatment has caused an increased diuresis but no change in the proteinuria plasma proteins or plasma lipids and finally type D that the treatment has been completely without effect The various types of response are shown in fig 1 which also illustrates an important point that in cases of type A or type B response the effect is observed quite suddenly and usually within about 15 days of the start of treatment Only such reactions to treatment should be ascribed to the glucocorticoids In those cases in which there is increase in diuresis and disappearance of or reduction in the proteinuria after longer (weeks or months) treatment with small doses of glucocorticoids the effect should not be ascribed to the drug but is more probably a spontaneous remission

It is difficult to determine the incidence of spontaneous remission in the primary nephrotic syndrome on the basis of published material among other things because of the different observation times On the basis of the available literature it must however be concluded that the spontaneous incidence of type A and B response is of the same order and in the region of 20 %, i e the sum of the type A and B responses to a given treatment must exceed about 40 % before any definite effect can be ascribed to the treatment The different materials are often not directly comparable because of the use of different preparations in varying doses Whilst the side effects of the various preparations are both qualitatively and particularly quantitatively different it has however become apparent that the therapeutic effect is on the whole identical when the preparations are given in equivalent doses (1)

As regards the dosage and the duration of the treatment there has been a tendency over the years to increase both with improvement in the results Today the majority of physicians would recommend a prednisone dosage of about 1 mg/kg/day or equivalent doses of the other preparations The high dosage is maintained until improvement is observed or for at most four weeks If this treatment leads to a type A or B response the dosage is then reduced to a maintenance dosage of 15–20 mg prednisone daily (for an adult) and this dosage is given for one year after which it is attempted to withdraw the prednisone gradually In cases with type C or D response the glucocorticoid is withdrawn after the four weeks

There has been discussion as to whether after four weeks of ineffective treatment with glucocorticoids in the above mentioned doses the dosage should be increased to for example the double Some workers do not consider that anything is gained by this Our experience is on the whole in accordance with this However there are a few patients in whom on the basis of the investigations carried out (see below) it is to have been expected that there would be a beneficial effect which has not become manifest and

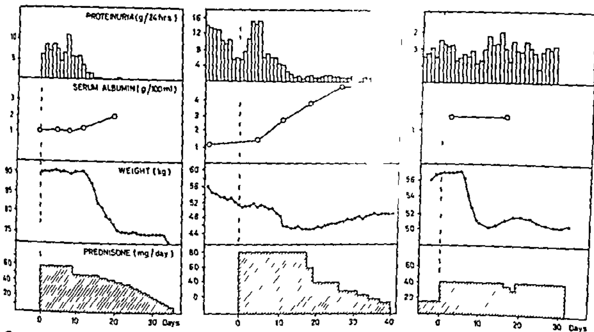


Fig 1 Examples of type A B og C response (from left to right) For details see text

in these cases a higher dosage should be tried In these cases we use about 2 mg prednisone/kg/day for a further four weeks

Over the years experience has been gained as to which of the primary nephrotic syndromes show the best reaction to glucocorticoids Renal biopsy has been of particular importance in this connection It has thus been demonstrated in several investigations that the fewer changes found on light microscopy of renal tissue the more effective the treatment The best results are achieved where there are few or no histological changes in the glomeruli In cases with definite glomerular changes some workers have found these drugs most effective in proliferative others in membranous glomerulopathies (for references see 5) In a similar way, investigation of the glomerular passage of plasma proteins has been of importance in the selection of those cases of nephrotic syndrome in which glucocorticoid therapy may be expected to give good results Without going into details it may be mentioned in brief that the glomerular passage of plasma proteins may be selective or non selective In selective passage it is especially the plasma proteins with low molecular weights (e g albumin) which pass through the glomerular membrane whilst non selective passage is characterized by the equally easy passage of all plasma proteins regardless of molecular weight It has become apparent that the more selective the glomerular passage the better the response to glucocorticoids (for references see 5)

The following review of the results of glucocorticoid treatment of the nephrotic syndrome is to a great extent based on a report by Adams et al (1) from 1962 These workers collected from the literature the results of glucocorticoid treatment in 1,200 patients with nephrotic syndrome The results of treatment of 771 cases of nephrosis in children will be described first In 366 cases the treatment lasted for less than two weeks As may be seen from table I type A and B responses were observed in 13 % and 11 % of the children respectively The average observation time was eleven months The remaining 405 children were treated for three to four weeks and in some cases the treatment was extended with maintenance therapy In this group type A and B responses were found in 54 % and 21 % of the children respectively i e the percentage of A responses was about four times as high as in the first named group The average observation time in the latter group was 20 months (table II)

The long term prognosis may be seen from fig 2 which is taken from Riley (13) It is apparent that the four year survival in 554 children treated with glucocorticoids was 77 % as compared with a survival rate of 61 % in 318 children with nephro[illegible] who were not treated with glucocorticoids The difference is statistically significan[illegible] There would thus seem [illegible]

S JMMARY OF RESPONSES TO CORTICOSTEROID THERAPY IN NEPHROTIC SYNDROME DUE TO PRIMARY RENAL DISEASE

SHORT TERM THERAPY (Usually < 2 weeks) (Follow up 1 64 months average 11 months)

No of Patients		Response classificat on A	B	C	D	Deaths
543		69 (13%)	55 (10%)	289 (53%)	118 (24%)	70 (13%)
Ch ld en	366	46 (13%)	39 (11%)	206 (56%)	75 (20%)	40 (11%)
Adults	107	17 (16%)	15 (14%)	32 (30%)	43 (40%)	21 (20%)

(F om Adams et al 1962)

Table I Results of glucocorticoid therapy in 543 patients with nephrotic syndrome

In some materials no distinction is made between children and adults and the total number of patients is not therefore identical with the sums of the various columns For further details see text

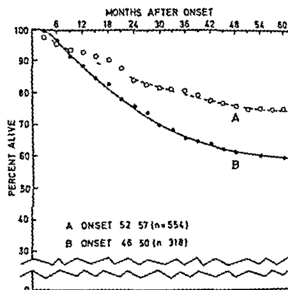


Fig 2 Survival graphs for children with nephrotic syndrome
o - - - o glucocorticoid therapy
•——• no glucocorticoid therapy

be no doubt that treatment with glucocorticoids has improved the prognosis of the nephrotic syndrome in children

The results of treatment of the nephrotic syndrome in adults are not so unequivocal Table II shows the results of glucocorticoid therapy in 157 adult patients The average observation time was 20 months Type A and B responses were obtained in 21 °/₀ and 22 °/₀ of cases respectively These figures are almost identical with the spontaneous incidence

SUMMARY OF RESPONSES TO CORTICOSTEROID THERAPY N NEPHROTIC SYNDROME DUE TO PRIMARY RENAL DISEASE

PROLONGED THERAPY (3 4 weeks or more w th or without maintenance) (Follow up 1-90 months average 20 months)

No of Patients		Response class fication A	B	C	D	Deaths
657		293 (44 5%)	157 (24%)	96 (14 5%)	111 (17%)	61 (9%)
Ch ldren	405	218 (54%)	85 (21%)	42 (10%)	60 (15%)	28 (7%)
Adults	157	33 (1%)	35 (22 3%)	46 (29 3%)	43 (27 4%)	31 (20%)

(From Adams et al 1962)

Table II Results of glucocorticoid therapy in 657 patients with nephrotic syndrome

In some materials no distinction is made between children and adults and the total number of patients is not, therefore identical with the sums of the various columns For further details see text

of type A and B response mentioned above Later investigations (4 5, 8, 10 12 14 17) revealed type A + B responses in from 15 °/₀ to 61 °/₀ of cases (table III)

There may be many explanations of these very varied results Varying numbers of renal biopsies have been carried out in the different materials This implies that some of the cases of nephrotic syndrome which on clinical grounds were classified as primary might in fact, have been secondary, which for the first comprise a larger percentage of the cases of nephrotic syndrome in adults than in children and secondly only rarely react positively to glucocorticoid therapy Similarly the distribution of the different histological types which are known to react differently to treatment may have varied However it is apparent from table III that this cannot be the whole explanation and in summary it can only be said of glucocorticoid therapy in the nephrotic syndrome in adults that despite the fact that some more recent investigations indicate that the treatment may be effective it would seem necessary to carry out a prospective study of a large number of patients who are investigated and treated in the same manner before anything definite can be said about the importance of the glucocorticoids in this connection

Where the side-effects of glucocorticoid therapy are concerned only two of these will be specially

Authors	Numbe r patients	Type f re ponse %			K d y biopsy % of patients	Pe cent of k dney biopsies show ng minimal hang	A e ge t m of f low p m h
		A	B	A B			
Iversen et 1 1963	42	17	14	31	52	0	39
Pr rl et 1 1963	27	11	4	15	100	33	?
Res & Smith 1963	26	15	27	42	54	0	24
Gol l al 1964	26	8	31	39	50	8	13
Traege et al 1965	49	14	37	51	100	13	I 1 at po
Rose et al 1966	21	5	24	29	?	?	In 1 at respon
Jensen & Jensen 1967	23	29	22	61	83	32	1 1 respon

Table III More recent data on the glucocorticoid ther ipy of primary nephrotic syndrome in adults

mentioned whilst reference is made to the other articles in this symposium for discussion of the remainder Where there is a positive effect of gluco corticoid therapy there will occasionally be an enormous increase in the diuresis with consequent fall in the plasma volume An example of this is shown in fig 3 Hypovolaemia often leads to ortho static hypotension which may occasionally give rise to thrombosis formation This implies that espe cially in older patients it may be necessary to ad

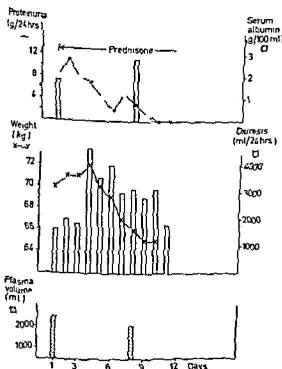


Fig 3 Changes in serum albumin weight diuresis and pla ma volume in type A response to glucocorticoid therapy in the nephrotic syndrome

mu
gl
T
th
ch
T
it
ke
str
studi
mat rial w h we
who h d eceive d
found in these case
ted in patients who ha been treated with gluco corticoids for rea ons other than the nephrotic syndrome Their significance is unknown (3 6)

DISCUSSION

Dr Loren en Nephrology seems to represent another of the fields in which steroid therapy has altered the prognosi or certain diseases Have there been any studies which have revealed how long it is necessary to continue treatment with steroids in the nephrotic syndromes when the treatment is effective? Is it pos ible to be guided by proteinuria and to withdraw the treatment once the proteinuria has disappeared or should treatment be continued for some time after this?

Dr Jensen Insofar as I am aware this has not been investi gated As I mentioned we continue treatment for about one year after the patient s urine has become protein free

Dr Bind r I would like to comment on the marked increase in diuresis following prednisone therapy in some of these patients Some reports ha e been published recently which suggest that pharmacological doses of the glucocorticoids cause an inhibition via the hypothalamus of secretion of the antidiuretic hormone

Dr Loren en Is the main conclusion with regard to steroid treatment of glomerulonephritis that this therapy has no effect on the course of the illness unless the nephropathy is part of a connective ti sue disease?

Dr Jensen Yes this would seem to be the case on the basis of what little information is to be found in the literature On the other hand as far as I know there have been no controlled studies of the effect of treatment with the glucocorticoids on acute subacute and chronic glomerulonephritis

REFERENCES

1 Adams D A Maxwell M H & Bernstein D Cor ticosteroid therapy of glomerulonephritis and nephrotic syndrome A Review J Chron Dis 15 29–50 1962
2 Armstrong S H & Kushner D S Current status of steroid therapy in chronic glomerulonephritis in the adult The unnatural history of Bright s disease Amer J Med 29 377–385 1960

3 Bou ssou H Fam liades J & Rakotondra n be A Les les ons glomérula res provoquees par la cort co hé rap e Étude exper mentale J Urol Néphrol 70 782 784 1964
4 Gotze H McCaughey W T E & Womersley R A Stero ds in treatment of the nephrot c synd ome n adults Br t med J 5379 351 354 1964
5 *Jensen H & Jensen E Stero d treatment of the nephrot c* syndrome in adults Acta med scand 18? 741 754 1967
6 K nosh ta Y Fuj saki S M kam A Mor ta T Ich nose M Arakawa M & Ebe T Stero d nephro pathy A case of renal d sease show ng s m lar changes of l po d nephros s mod fied by stero d treatment Acta med b ol (N gata) 14 51–65 1966
7 Lorenzen I B un C & V debæk AA *Lupus neph tis* treated w th a cytostat c agent A case of sys em c lupus erythematosus w th lupus nephr t s treated with 6 mer captopur ne Dan med Bull 13 151 154 1966
8 Nesson H R Sproul L E Jr Relman A S & Schwartz, W B Adrenal stero ds n the treatment of d opath c nephrot c syndrome n adul s Ann ntern Med 58 268 277 *1963*
9 Oppenhe me E H & Este ly J R Glome u ar les ons *in the nephrot c syndrome and the r relat on to cort sone* therapy Bull Johns Hopk Ho p 113 158 172 1963
10 Pearl M A Burch R R Cawa al E McCracken B H Woody H B & S e nbe g W H Nephrot c synd ome A cl n cal and patholog cal *study Arch* ntern Med 112 716–726 1963
11 Pollak V E Piran C L & Kark R M Effect of large doses of predn sone on the renal les ons and l fe span of pat ents w th lupus glomerulonephr tis J Lab cl n Med 57 495 511 1961
12 Poser G Kluthe R & Adler P Zur Therap e des *neph otischen Syndroms Med Kl n 61 432–435 1966*
13 R ley C M Stat st cal evaluat on of survival of neph o t c ch ldren treated w th stero ds In Metcoff J (Ed) P oceed ngs of the 10th annual conference on the nephro t c syndrome N Y Nat onal Kidney D sease Founda t on 1958 p 273
14 Ross E J & Sm th J F The use of ste o ds n the treatment of the nephrotic syndrome n adu ts Quart J Med 32 65 77 1963
15 S *menhoff M L & Merr ll J P The spectrum of* lupus nephr t s Nephron 1 348 374 1964
16 Thorn G W Merr ll J P Sm th S Roche M & Frawley T F Cl n cal stud es w th ACTH and cort sone n renal d sease Arch ntern Med 86 319 354 1950
17 Traeger J Fr es D Brunat N & Franco s B *Resultats de la cort cothérap e dans les syndromes* néphrot ques prim t fs de l adulte Lyon med 214 1131 1185 1965

Acta med scand Suppl 500 pp 67-69 1969

GLUCOCORTICOIDS IN INFECTIOUS DISEASES

by
Poul Effersoe

During the past 20 years the period during which the glucocorticoids have been used therapeutically there has been changes in opinion about their use in *infectious* diseases At first i e at the end of the 1940s they were considered on evidence from animal experiments to be absolutely contra indicated in all infections Later during the 1950s there were reports of dramatic effects on the signs and symptoms in severe infections which led to unfounded optimism

It was not until the present decade that it became possible to distinguish between dramatic masking of signs and symptoms and an actual effect on the course of the disease The reason for the uncertainty with regard to the effect of the glucocorticoids on infectious diseases is that these hormones have both a beneficial and a deleterious effect in infections and it is not possible to predict which effect will be dominant in any given situation Unfortunately there are still a large number of problems connected with glucocorticoids and infectious diseases which have not been solved by the use of controlled trials

BASIS FOR THE USE OF GLUCOCORTICOIDS IN INFECTIOUS DISEASES

The tissue reactions induced by the micro organism in the host organism can be inhibited by the glucocorticoids

The antitoxic and anti allergic effects of the glucocorticoids are an indisputable advantage to the host organism whereas the effect on the defensive mechanisms of the body is unfortunate

A reduction of the inflammatory phenomena may lead to a relief of symptoms, but the defensive mechanisms are at the same time inhibited among other things by the reduction of vascular permeability such that there is inhibition of the exudation of antibacterial and antitoxic antibodies and of the natural defensive substances The migration of leucocytes from the vessels and the phagocytic abilities of the leucocytes are reduced whilst the synthesis of interferon is similarly reduced The glucocorticoids mask the symptoms by the inhibition of the symptoms of inflammation but they may also alter a host parasite relationship in favour of the parasite In bacterial infectious diseases it is therefore a prerequisite for the enjoyment of the beneficial anti toxic and anti allergic effects of the glucocorticoids that *other methods i e bacterial chemotherapeutic* drugs are employed to compensate for their inhibitory effect on the body s defensive mechanisms

In diseases caused by viruses in which in contrast to the bacterial diseases no effective antimicrobial drugs are available to compensate for the inhibition of the defensive mechanisms the glucocorticoids are contra indicated in the phase of active division of the virus but under special circumstances they may be indicated in the phase in which the symptoms are due to secondary allergic or auto immune reactions

INDICATIONS FOR THE USE OF GLUCOCORTICOIDS IN INFECTIOUS DISEASES

In infectious diseases the glucocorticoids are used partly as substitution therapy and partly on account of their anti inflammatory effect

Substitution therapy is indicated in meningococcal sepsis with adrenal damage (Waterhouse Friderichsen s disease) and in the treatment of infectious diseases in patients with Addison s disease or in patients receiving long term steroid therapy in which cases it is necessary to administer the glucocorticoids in doses which at least correspond to the normal maximum production of the adrenals In meningococcal sepsis very large doses of glucocorticoids are given in order to inhibit the effect of the meningococcal toxin on the vessels and to prevent shock

The exploitation of the anti inflammatory effect of the glucocorticoids is based on either their antitoxic or their anti allergic actions

The antitoxic effect is not due to any neutralization

of the toxins but to a reduction in the sensitivity of the tissues to toxin This action is exploited in septic shock caused by for example Gram negative rods in which large doses of glucocorticoids restore the sensitivity of the endotoxin damaged vessels to the sympatheticommetics especially noradrenaline Occasionally such toxic shock is iatrogenic For example during the treatment of severe typhoid fever with chloramphenicol a Herxheimer reaction with shock may occur during the first days of treatment due to the release of large quantities of endotoxin from the typhoid bacteria which are affected by the antibiotic In treatment of the most severe cases of typhoid fever it is therefore indicated to give glucocorticoids together with antibiotics at the beginning of treatment (10)

In control trials at Blegdam Hospital Copenhagen it has been demonstrated that the mortality rate in pneumococcal meningitis is reduced if glucocorticoids are administered together with the antibiotic therapy and there are reasons to assume that this is due to an action on the bacteraemic shock (6)

Recommendations have been made for the use of the antitoxic effect of the glucocorticoids in other serious conditions which are ascribed to toxins There have been descriptions of dramatic improvement in the clinical state in many diseases but it is *not known whether there is any beneficial effect on* the course of the illness as a whole (9) The use of glucocorticoids in patients with mononucleosis who are only moderately ill in order to achieve a masking of the symptoms is abuse of these hormones when it is taken into consideration that the mortality rate in this condition is almost nil and that it is extremely rare for there to be any permanent sequelae

The anti allergic action of the glucocorticoids is exploited in infectious diseases caused not only by virus and bacteria but also by metazoa and protozoa

The administration of glucocorticoids together with antibiotic therapy in the exudative phase of miliary tuberculosis or tuberculous meningitis has a definite beneficial effect In Blegdam Hospital the introduction of glucocorticoids in the treatment of tuberculous meningitis led to a fall in the lethality (8)

It is difficult to demonstrate any beneficial effect on pseudocroup presumably because allergy plays a part in only a proportion of cases

Among the tropical diseases the anti allergic action of the glucocorticoids has been exploited successfully in the treatment of life-endangering black water fever, which is due to an auto immune reaction and of the allergic reactions which may arise in connection with the institution of treatment in leprosy and filariosis (7) It is of great interest that it has been found possible to give patients with penicillin allergy the necessary penicillin therapy in for example endocarditis by means of the concomitant administration of very large doses of glucocorticoids (5)

Glucocorticoid therapy is effective in Steven Johnson s syndrome and those haematological complications of infectious disease which are due to allergic reactions This therapy can be used when any possible primary infectious disease can be treated with chemotherapeutic drugs or where it *has subsided Whilst controlled trials have demon*strated conclusively that the glucocorticoids have a beneficial effect on herpes zoster (2) herpes simplex of the eye is an absolute contra indication to their administration

DUBIOUS INDICATIONS

From theoretical considerations the glucocorticoids have been used in parotitis orchitis (mumps orchitis) as it was anticipated that an inhibition of the inflammatory reaction within the extremely unyielding capsule would reduce the incidence of pressure *atrophy of the testes The best of the controlled trials* have however revealed that this theoretical possibility did not work in practice (4 9)

The use of glucocorticoids in virus hepatitis is described by Quaade elsewhere in this symposium

In post infectious and post vaccination encephalitis which are very probably of allergic aetiology the glucocorticoids should theoretically have a beneficial effect but the preliminary reports of controlled studies would seem to indicate that the effect is in fact deleterious (1) The same is true of polyradiculitis

There seemed to be cause for alarm when it was discovered that such an innocent disease as varicella had a lethality rate of 12 % in children who were receiving long term glucocorticoid therapy for for example haematological diseases or asthma At one time this was ascribed to the glucocorticoids and it was attempted to withdraw the hormones when these children were exposed to varicella However more recent studies have revealed that the mortality rate in children with these disorders who are not receiving glucocorticoid therapy is also 12 % because of the basic disease (3)

CONCLUSION

Where the use of glucocorticoids in infectious diseases is concerned it is as a general rule possible to obtain an apparent improvement and occasionally a dramatic improvement due to the masking of symptoms but the course of the disease as a whole is unaltered The only definite indications for the use of glucocorticoids where these are not required as pharmacological substitution therapy are bacteriaemic shock pneumococcal meningitis exudative tuberculosis allergic reactions during the treatment of typhoid fever leprosy and filariosis attacks of black water fever in herpes zoster, and when it is found requisite to give penicillin to patients with penicillin allergy

PROPHYLAXIS AGAINST INFECTIONS

In conclusion brief mention will be made of prophylaxis against infections in patients receiving glucocorticoid therapy In general the prophylactic administration of antibiotics is valueless Infections occur just as frequently and are merely caused by micro organisms which are resistant to the antibiotic concerned There is one exception to this rule If it is necessary to administer glucocorticoids to a patient with a pos ible latent tuberculosis isoniacid should be given at the same time This inhibits the reproduction of tuberculosis bacteria and no resistant strains can therefore arise

Control cultures during glucocorticoid therapy for example weekly blood cultures are of very little value The only means of discovering masked infections is by careful attention to all symptoms even when the patient is afebrile The most important prophylaxis against the resurgence due to glucocorticoids of latent infections consists of not misusing the glucocorticoids in cases in which they are not definitely indicated and where they are used of administering them for short periods only

DISCUSSION

Dr Loren en In recent years there seems to have been a considerable change in attitude towards the use of glucocorticoids in infectious diseases During the first years after the introduction of these agents their use in infectious diseases was considered to be contra indicated It would now seem that in certain infectious diseases the glucocorticoids can lead to a marked improvement in prognosis

Dr Bennike I cannot agree with Effersøe that the indication for using glucocorticoids in bacteriaemic shock and pneumococcal meningitis has been established Opinions still differ considerably with respect to the value of this treatment in these conditions We have previously discussed the use of glucocorticoids in ulcerative colitis In this connection it should be mentioned that during steroid therapy there may be a severe aggravation of amoebic dysentery This should be borne in mind as patients with amoebic dysentery now visit Denmark frequently

Dr Lorenzen Have any investigations been carried out in human clinical practice to determine whether high doses of glucocorticoids reduce the lethality of septic shock?

Dr Effersøe No not that I know of

Dr Loren en Would you still use glucocorticoids in septic shock?

Dr Effersøe Yes because I consider that their use is theoretically well founded and their beneficial effect has been well-established in animal experiments

Dr Lorenzen The fall in the mortality rate in tuberculous meningitis is surprising Was this a controlled investigation?

Dr Effersøe It was a consecutive study in which the untreated group temporally directly preceded the treated group

Dr Bruun What dose of corticosteroid would you give to a patient with penicillin allergy who had to be treated with penicillin?

Dr Effersøe I would as far as possible avoid the use of penicillin in penicillin allergy This is usually possible thanks to the wide choice of chemotherapeutic drugs

REFERENCES

1 Bøe Johs Solberg C O & Sæter T Corticosteroid treatment of acute meningoencephalitis A retrospective study of 346 cases Brit med J 1 1094–1095 1965
2 Elliott F A Treatment of herpes zoster with high doses of prednisone Lancet 2 610–611 1964
3 Falliers C J Ellis E F & Bukantz S C The course and management of varicella in children receiving steroids for intractable asthma Sth med J 57 1054–1058 1964
4 Klemola E & Somer P Corticotrophin och cortison vid Parotitorchit Nord Med 56 1128–1129 1956
5 Raper A J & Kemp V E Use of steroids in penicillin sensitive patients with bacterial endocarditis New Engl J Med 273 297–300 1965
6 Rosdahl N Jensen K & Ranek L Kortikosteroid behandling af pneumokokmeningitis Nord Med 79 146–150 1968
7 Spink W W ACTH and adrenocorticosteroid as therapeutic adjuncts in infectious diseases New Engl J Med 257 979–983 and 1031–1035 1957
8 Søltoft J Late prognosis in tuberculous meningitis Scottish Scandinavian Conference on Infectious Diseases Edinburgh Glasgow 1965
9 Weinstein L Corticotrophin and corticosteroids in human viral infections Med Clin N Amer 46 1141–1161 1962
10 Woodward T E & Smadel J E Management of typhoid fever and its complications Ann intern Med 60 144–157 1964

Acta med scand Suppl 500 pp 71-75 1969

THE FUNCTION OF THE PITUITARY GLAND AND THE ADRENAL CORTEX DURING TREATMENT WITH GLUCOCORTICOIDS

by
Christian Binder

The normal regulation of cortisol secretion

In the normal individual there is a close co ordination between the central nervous system the adenohypophysis and the adrenal cortex The adrenal cortex does not contain secretory nerve fibres and the spontaneous secretion of cortisol from this gland is very limited The direct stimulus for the secretion of cortisol is corticotrophin (ACTH) The release of ACTH from the adenohypophysis is controlled by the hypothalamus in which there is a varying density of cells in which an ACTH releasing agent (CRF) is produced This is released under certain circumstances into a portal venous system which surrounds the pituitary, which is thereby stimulated to release ACTH There are many reasons to suppose that in the intact individual the entire regulation of the cortisol production of the adrenal cortex is mediated via the hypothalamus (28) In principle the hypothalamus acts as a control station which processes all impulses both activating and inhibitory which have any influence on the secretion of cortisol from the adrenal cortex

There are three variables which are characteristic of this system diurnal rhythm correlation between the plasma concentration of cortisol and the secretion of ACTH and the reaction of the system to various forms of stress

The diurnal variation in the plasma concentration of cortisol in a normal unstressed person shows only small individual variations

The highest value is found during the morning subsequently there is a fall lasting until about midnight when the lowest value is reached This diurnal rhythm is preceded by a corresponding variation in the plasma concentration of ACTH (18 20) Following operation on certain parts of the hypothalamus and after hypophysectomy this diurnal variation is abolished (28) In contrast it is found that the diurnal variation of the ACTH concentration in the plasma is preserved in patients with untreated Addison s disease (12) In these patients the ACTH concentration in the plasma is raised This illustrates another of the variables named above the correlation between the plasma concentration of cortisol and the secretion of ACTH A fall (or an increase) in plasma cortisol concentration in excess of that which is a function of the diurnal variation will initiate an increased (or decreased) release of corticotrophin

The sensitivity of the system to an increase in the plasma cortisol concentration varies throughout the 24 hours Following the administration of 0 5 mg fluormethylprednisolone (DECADRON ®) at 8 a m on two consecutive days to normal individuals there was only a slight fall in the plasma concentration of cortisol and the diurnal rhythm was preserved The 24 hour cortisol secretion was reduced by 60 % Following administration of the same dose at midnight on two consecutive days the plasma cortisol concentration remained low and the 24 hour cortisol secretion was reduced by 90 % (20) During constant infusion of DECADRON from 10 p m to 12 noon in normal individuals the diurnal rhythm was preserved but there was a reduction in cortisol production with increasing infusion rate up to 0 02 mg per hour corresponding to a total dose of 0 28 mg Following infusions of 0 05 mg per hour (total dose 0 70 mg) the cortisol production was blocked (5)

The normal rhythm in the system can thus be preserved by suitable timing of the administration of pharmacological amounts of glucocorticoid corresponding to 0 5 mg fluormethylprednisolone = 3½ mg prednisolone = 17 mg cortisone = 20 mg cortisol

Both inappropriate administration with regard to the time of administration and larger doses will inhibit the secretion of ACTH to such an extent that there is first atrophy of the [illegible] later

approaching When this occurs the patient should be admitted for observation and glucocorticoid therapy should be instituted only when there is evidence of incipient adrenal cortical failure

In the majority of patients who are receiving glucocorticoid therapy operation will initiate an adequate increase in the plasma cortisol concentration (16) Two out of sixteen patients did not show any such increase but nonetheless did not show a fall in blood pressure during the operation

If it is possible for the patient to be closely watched by trained personnel then it is justifiable to refrain from administering extra glucocorticoid to these patients but as soon as there is the slightest uncertainty as to whether failure might be beginning treatment should be instituted with the intravenous administration of a water soluble cortisol preparation If on the other hand there is the slightest doubt as to whether it is possible to offer the patient an adequate observation, then the classical lines of treatment should be followed

In conclusion it may be mentioned that alternating glucocorticoid therapy, in which one or more days without treatment are inserted in the therapy would seem to reduce the suppression of the normal cortisol regulation even after a longer period of treatment (1 11, 14 25) It is outside the scope of the present paper to describe the special form of glucocorticoid therapy which is achieved by the administration of ACTH This type of therapy will naturally not lead to adrenal cortical atrophy, but the risk of an inhibition of the hypothalamus pituitary must remain unchanged

DISCUSSION

Dr Loren en It is surprising that the clinical importance of failure of adrenal cortical and pituitary function due to long term steroid therapy has only been demonstrated in a few patients I presume that this is mainly due to the fact that this problem has not been sufficiently studied

Dr Binder From a study of the literature I have been able to find only a few cases in which it could be proved that there was a failure of adrenal cortical function In the innumerable other case histories which have been published the authors have postulated adrenal cortical failure as an associated factor in the serious complications This has been either on the basis of an adrenal cortical atrophy demonstrated at autopsy or from the clinical picture especially a dramatic effect of glucocorticoids However there is no definite correlation between the degree of adrenal cortical atrophy and the degree of reduction of function and a dramatic clinical effect following the administration of glucocorticoids is not proof of adrenal cortical failure

Dr Loren en On the other hand it is probably true to say that it has been convincingly demonstrated that long term administration of the glucocorticoids leads to a reduction in adrenal cortical function and that it is known from other diseases e g Addison s disease that this increases the risk of serious possibly even life-endangering complications

Dr Binder I would like to draw it up sharply and say that we know that more than 35 months of treatment with glucocorticoids in doses corresponding to more than 15 mg prednisone daily definitely lead to suppression of adrenal cortical function and thereby to considerable risk of serious complications in any stressful situation

Dr Loren en Isn t the period you name to some extent merely an expression of what has been studied? Isn t there a shortage of investigations into the effects of short term treatment and smaller doses?

Dr Binder Yes

Dr Andersson Is it your opinion that supplements of glucocorticoids should not be given in all situations of acute stress in patients receiving long term glucocorticoid therapy? Does it not increase the risk that such treatment will be too late if nothing is given prophylactically? A second question In long term therapy should we not change to intermittent dosage such that cortisone or prednisone is given during the phase in which the cortisol concentration in the blood is increasing spontaneously i e in the morning?

Dr Binder If it is possible to observe the patient sufficiently carefully and to give supplements of water soluble cortisol preparations immediately should there be any fall in blood pressure then I consider that it is possible to avoid the routine administration of glucocorticoids in situations of acute stress With reference to th value of intermittent administration of glucocorticoids in the hope of influencing the pituitary and adrenal cortical function to a lesser extent all that can be said is that this problem has as yet not been sufficiently elucidated

Dr Hvidberg If the daily dose of glucocorticoids exceeds an amount corresponding to 15–20 mg prednisone then it is in any case useless to use intermittent therapy When lower doses are used one should probably try intermittent therapy but as Dr Binder has said there is as yet no evidence of the value of this

Dr Lou What is the maximum amount of cortisol which is released from the adrenals on acute stress and what is the relation between this amount and the very large doses of glucocorticoids which are given as supplements during such acute situations?

Dr Binder The endogenous release of cortisol rarely exceeds 100 mg in 24 hours and this is considerably less than the doses which are generally used as supplements

Dr Hvidberg In this connection it should be borne in mind that a large part of the amount of glucocorticoid which is injected is bound to the serum albumin such that the con centration of the free active hormone is lower than would be expected

REFERENCES

1 Adams D A Gold E M Gonick H C & Maxwell M H Adrenocortical function during intermittent cor ticosteroid therapy Ann intern Med 64 542–551 1966

2 Amatruda, T T Hollingsworth D R D Esopo N D Upton G V & Bondy P K Study of the mechanism of the steroid withdrawal syndrome J clin Endocr 20 339–354 1960

3 Andersson E & Kjerulf K Adrenal cortical function during steroid therapy Acta med scand 169 577–582 1961

4 Bierich J R Kersten I & Maruektad S Plasma cor ticosteroids and their responsiveness to corticotrophin after long term therapy with corticosteroids and corti cotrophin Acta endocrin (Kbh) 31 40–48 1959

5 Ceresa F Strumia E Angeli A & Dellepiane M Behaviour of the feedback mechanism controlling ACTH secretion in normal subjects and under different endo crine conditions and relative clinical implications Pro ceeding of the 2nd International Congress of Endocri nology London pp 1027–1040 1964

6 Cope C L Adrenal steroids and disease Pitman Medical Publishing Co London p 313–343 1964

7 Cope C L Some adrenal facts and fancies Proc roy Soc Med 58 55–61 1965

8 Danowski T S Bonessi J V Sabeh G Sutton R D Webster M W & Sarver M E Probabilities of pitui tary—adrenal responsiveness after steroid therapy Ann int Med 61 11–26 1964

9 Elb S & Jensen J K Pla makortisolbestemmelse hos kortikosteroidbehandlede operationspatienter Nord Med 76 1210–1214 1966

10 El Shaboury A H Adrenal and pituitary function in asthmatic patients receiving continous long term steroid therapy Thesis University of Wales 1966

11 Fleisher D S & Pellecchia P Pituitary—adrenal responsiveness after corticosteroid therapy in children with nephrosis J Pediat 70 54–59 1967

12 Graber A L Ney R L Nicholson W E Island D P & Liddle G W Natural history of pituitary—adrenal recovery following long term suppression with corticosteroids J clin Endocr 25 11–18 1965

13 Grant Sylvia D Forsham P H & DiRaimondo V C Suppression of 17 OHCS in plasma and urine by single and divided doses of triamcinolone New Eng J Med 273 1115–1118 1965

14 Harter J G Reddy W J & Thorn G W Studie on an intermittent corticosteroid dosage regimen New Eng J Med 269 591–596 1963

15 James V H T & Landon J The investigation of adreno cortical insufficiency Triangle 7 320–325 1966

16 Jørgensen A W Hypofyse binyrebarkfunktionen und r langtidsbehandling med kortikosteroider Ugeskr Læg 127 44–47 1965

17 Kyle L H Meyer R J & Canary J J Mechanism of adrenal atrophy in Cushing s syndrome du to adre nal tumor New Engl J Med 257 57–61 1957

18 Landon J James V H T Wharton Margaret & Friedman M Threshold adrenocortical s nsitivity in man and its possible application to corticotrophin b o assay Lancet II 697 700 1967

19 Moore F D Hormones and stress Endocrine chang s after anaesthesia surgery and unanaestheti ed trauma n man Recent Progr Hormone Res 13 527–553 1957

20 Nichols C T & Tyler F H Diurnal varia ion in adrenal cortical function Ann Rev Med 18 313–324 1967

21 Robinson B H B Mattingly D & Cope C L Adrenal function after prolonged corticosteroid therapy Brit med J I 1579–1584 1962

22 Salassa R M Bennett, W A & Keating F R Post operative adrenal cortical insuff iency occurrenc in patients previously treated with cortison J Amer med Ass 152 1509–1515 1953

23 Sandberg A R Erik Nes K Migeon C J & K ept G F Plasma 17 hydroxycorticost roids in hyperfun tion suppression and deficiency of adrenal cortical fun tion J Lab clin Med 50 286–296 1957

24 Savage O The place and problems of corticosteroid therapy Brit J clin Pract 19 657–665 1965

25 Soyka L F Treatment of the nephrotic syndrome in childhood Amer J Dis Child 113 693–701 1967

26 Spaethe P Minneker C & Otto C Beurteilung der Nebennierenrindenfunktion unter Glucocorticoidbehand lung mit Hilfe des Synacten Kurztests Dtsch Med Wschr 91 1483–1490 1966

27 Treadwell B L J Savage O Sever E D & Cope man W S C Pituitary—adrenal function during corticosteroid therapy Lancet I 355–358 1963

28 Yates, F E Physiological control of adrenal cortical hormone secretion in Eisenstein A B The adrenal cortex by 26 authors J A Churchill Ltd London 1967 pp 133–183

Acta med scand Suppl 500 pp 77-80 1969

UNDESIRABLE EFFECTS OF GLUCOCORTICOIDS

by
Flemming Quaade

My motive for deliberately avoiding the word side effects in the title is explained by the special position occupied by corticosteroids in therapy True side-effects may occur with all potent drugs either in the form of unpredictable reactions due to allergy or low tolerance in the individual patient or as toxic phenomena following simple overdosage Naturally all such side-effects are causally related to the pharmacodynamics of the drug used and therefore to some extent to be expected but they are on the other hand so rare limited benign or avoidable that we do not think of them as an inherent part of the treatment

Examples of these side-effects include such varied phenomena as bradycardia and dyspepsia with digitalis anaemia with cytostatic drugs haemorrhage with anticoagulants and exanthema and staphylococcosis with penicillin The undesirable effects encountered during treatment with corticosteroids—apart of course from hormonal substitution therapy—are of a different nature

Whenever we decide to use corticosteroids outside the realm of mere substitution we have beforehand renounced any attempt to influence the aetiological agent directly Instead we attempt a different approach—to alter the mode of reaction of the organism to the noxious agent By deliberately administering to the patient unphysiologically high concentrations of corticosteroids in other words by establishing an iatrogenic hypercorticism our aim is to suppress certain reactions particularly in the connective tissue and the immunological apparatus which under normal circumstances are expedient and desirable but which are assumed to be maladjusted and injurious *in the illness under consideration* It is obvious that when this is the aim of the treatment and when the agent used is an almost universally effective drug such as a corticosteroid we cannot speak of side-effects in the meaning defined above but rather of effects which are to be *anticipated from the nature of the drug and the* method used although of course these effects are undesirable

It follows that both the decision to give a patient *steroid therapy and the ability to maintain this at* the right dosage means to balance the risks present and future associated with the untreated state against the inconveniences and dangers introduced by steroid treatment Furthermore once treatment has been started it may be very difficult to determine whether a new symptom should be ascribed to the treated disorder and its associated complications or whether we are dealing with an undesirable effect of the steroid There are many practical examples of this

If a patient receiving steroids for polyarthritis develops a peptic ulcer it may be steroid induced but it may also be due to the fact that the patient is taking salicylates or phenylbutazones for his arthritis It may also be due to an interplay of these ulcerogenic factors and finally it may merely be the *coincidental occurrence of two common diseases* ulcer and arthritis In the same category of patients decalcification of the skeleton may be a steroid effect or a consequence of the inactivity resulting from the illness Similarly patients with cirrhosis of the liver especially post menopausal women may develop osteoporosis even when they are not on steroid therapy In patients with cirrhosis whether they are receiving steroids or not the incidence of ulcer is higher than in the general population If a patient who is receiving steroids for ulcerative colitis develops an intestinal perforation it must be borne in mind that this complication might also have *occurred even if he had not received steroids It is* possible to mention many other examples which illustrate how difficult it may be to distinguish between a manifestation of the illness and a steroid effect and these questions which can only be finally answered by means of sufficiently large and con

Table I Undesirable effects of steroids in 207 patients with systemic lupus erythematosus (from Dubois 1966) Average dosage of steroids corresponding to 20-25 mg prednisone daily

	Paramethasone 51 pts (%)	Dexamethasone 50 pts (%)	Methylprednisolone 40 pts (%)	Triamcinolone 29 pts (%)	Prednisone and prednisolone 37 pts (%)
Cushingoid	63	60	52	45	49
Hirsutism	12	28	8	21	3
Acne	8	10	10	17	16
Striae	10	4	3	14	3
Ecchymoses	32	20	27	14	3
Oedema	6	28	5	3	6
Diabetes	4	4	0	3	6
Peptic ulcer	29	30	17	37	33
Myopathy	2	0	0	19	0
Psychoses	0	2	0	0	0

trolled therapeutic trials must be borne in mind if the steroids are not to fall into undeserved discredit

The undesirable actions of the steroids are well known and it is merely necessary to list them some — peptic ulcer and osteoporosis — have already been mentioned To these may be added diabetes, reduced resistance to infection capillary fragility, hypertension, mental disturbances, myopathy hypokalaemia cataract and — as the sign most regularly observed in patients markedly influenced by steroids — a Cushingoid appearance In children there may be retardation of growth and there is a well-documented report (4) of a raised incidence of intrauterine death in a material of women who had received steroid therapy during pregnancy Sodium retention with oedema is often mentioned but hardly a great problem since cortisone has been abandoned in favour of prednisone and similar derivatives For the sake of completeness it should be mentioned that another risk associated with steroid therapy is the dangerous shock which may follow stress or withdrawal of the drug

The likelihood of the steroids having undesirable effects is to a certain extent but not invariably, correlated to the size of the dose and the duration of the treatment In acute or transient illness and in exacerbations of chronic diseases, where the treatment can be withdrawn within a few weeks it is often found that even at high dosage there is a particularly favourable relation between the desirable and the undesirable effects so that in establishing the indications for treatment we are justified in ignoring the latter The situation is completely different in the long term treatment of chronic illness especially where large doses of steroids are required periodically

Table 1 illustrates the nature and incidence of undesirable effects in a large material of patients who had received long term therapy with a relatively high dose of various steroid preparations corresponding to 100 mg cortisone or 20-25 mg prednisone daily The table is taken from Dubois book on systemic lupus erythematosis (1) Between half and two thirds of the patients developed a Cushingoid appearance About one third developed peptic ulcer (for some reason the percentage was lower in the methylprednisolone group) Diabetes occurred in only 4 %. One notes the high incidence of myopathy in the group receiving triamcinolone, and is seems surprising that no muscular lesions developed during dexamethasone therapy It is also remarkable that Dubois does not include osteoporesis in his table, he states that this is a rare complication

Table II is included for comparison This originates from the liver research group in Copenhagen

Table II Complications in 337 patients with cirrhosis 170 of whom were treated with prednisone and 167 with placebo Average steroid dosage 10-15 mg prednisone daily

	prednisone group No patients	placebo group No patients
Incidence of peptic ulcer	40	39
Deterioration of dyspepsia in patients with present or previous ulcer	8	4
New ulcers arising during treatment	6	1
Death from ulcer	5	2
Deterioration in diabetes present before treatment	6	2
Diabetes arising during treatment	5	2
Fracture of spine arising during treatment	2	3

which has studied patients with cirrhosis of the liver on long term therapy (1–5 years) who received smaller doses than Dubois patients i e 10–15 mg prednisone daily

The incidence of ulcer was approximately the same as in Dubois material namely 25 % but it may be seen from the table that only a minority of these developed during the treatment and aggravation of symptoms from ulcers which were already present was less common in the steroid group than we had anticipated

With regard to the low incidence of diabetes the figures are of the same order as those in the American material but we had a few cases of the serious complication fractured spine due to osteoporosis The fact that the same was true of the placebo group provides food for thought

As already stressed it is worthwhile bearing in mind that the complications mentioned also occurred in the placebo group — in our material the incidence was such that there was no significant difference between treatment and placebo groups in these respects This was also true of deaths due to ulcers or sequelae thereof

A few remarks on the precautions which may be taken against the undesirable steroid effects

It is difficult to do anything for a Cushingoid appearance or an osteoporosis except to reduce the dosage By contrast it is usually easy to control a steroid provoked diabetes The steroid ulcers are most commonly prepyloric and they generally react satisfactorily to anti acids or reduction of dosage and it is by no means rare to observe spontaneous healing despite the continued administration of steroids

The steroid myopathy which closely resembles that which occurs in Cushing s disease rarely gives rise to therapeutic problems The most serious cases have been seen after treatment with the fluor substituted steroids and I think that these derivatives should be avoided On the whole it would therefore seem that one should not take too pessimistic a view of the undesirable effects of steroid therapy provided that the indications for the treatment are correct

In my view no single steroid disadvantage can be considered to form an absolute contra indication to a steroid therapy for which the indications are strong It must however be emphasized that whenever possible as in acute or transient conditions an attempt should be made to manage with a short course of treatment

In chronic diseases one should begin with a short trial with high doses in order to see whether the reaction is favourable If this is the case and one decides to use long term therapy the dose should be kept as low as possible, that is at a level which gives the maximum effect on the disease combined with a tolerable degree of undesirable effects

DISCUSSION

Dr Loren en I don t entirely agree with you when you emphasize that the side effects of the use of glucocorticoids are generally not v ry serious This is perhaps true of the majority of the ide effects which you have just mentioned but if the action on he adrenal cortical and pituitary function is included I would consider that the entire complex of side-effects is such as to call for circumspection in drawing up indications for the use of glucocorticoids Does the incidence of the various side-effects depend on which illness is treated?

Dr Quaade It is obvious that the inhibition of pituitary and adrenal cortical function is important This inhibition has probably also occurred in all the cirrhosis patients who have received long term therapy Nonetheless they do not die with a clinical picture which can be ascribed to failure of adrenal cortical function with a significantly greater frequency than the patients in the control material die with a similar clinical picture I therefore do not consider that this complication is of great importance

Dr Loren en In this evaluation of the side-effects it is important to emphasize that it is primarily based on the findings in one definite disorder cirrhosis of the liver which in itself has a rather high lethality

Dr Quaade It is quite true that there is a difference in the incidence of the steroid induced side-effects in different disorders thus the incidence of gastric ulcer shows a relatively greater increase during steroid treatment of SLE than during treatment of cirrhosis of the liver presumably because the incidence of ulcer in SLE is primarily lower than that in cirrhosis

Dr Binder There are two factors which I would like to emphasize in the evaluation of the side-effects produced by glucocorticoids in the material of the patients with cirrhosis of the liver The first is that the treatment has not been of particularly long duration when compared with that in other diseases such as for example rheumatoid arthritis In some reports of the latter the incidence of osteoporosis and spontaneous fracture has been found to be considerably higher than in your material

Secondly you have used relatively small doses of glucocorticoids 10–15 mg prednisone such that you are merely at the threshold of constant suppression of adrenal cortical function I therefore consider that one should be cautious in making generalization from the cirrhosis material In my opinion there is a considerable risk associated with treatment with glucocorticoids lasting for years at doses at the level of 15 mg prednisone or above

Dr Riis With regard to the so-called steroid induced ulcers in the stomach and duodenum it should be emphasized that there is little documented evidence of their causal relationship to glucocorticoid treatment There has been no reliably controlled investigation both with reference to the incidence of spontaneous ulcers in the different disorders and to the importance of high and low doses of glucocorticoids in the possible occurrence of ulcers It is improbable that small doses (10–15 mg prednisone) produce ulcers We know nothing certain about whether high doses do so

Dr Hilden Are anabolic steroids of any value as prophylaxis against steroid induced osteoporosis?

Dr Quaade In reply to Binder I will admit that many patients with rheumatoid arthritis have been treated longer than our cirrhosis patients However in my opinion there has been put forward no evidence even in these patients that the inhibition of pituitary and adrenal cortical function has increased the mortality rate With reference to the anabolic steroids no evidence has been brought forward in support of their prophylactic value in osteoporosis

Dr Olesen There is a thesis from Gothenborg (B Lindholm Acta allerg 22 1967 and Acta endocr (Kbh) 55 1–18 1967) which describes an investigation into the prophylactic effect of the anabolic steroids in glucocorticoid induced osteoporosis The patients suffered from bronchial asthma and received long term steroid therapy The result of this study was very interesting as it was found on measurements of the composition of the body that the most important effect of the anabolic steroids was to give the women the same total potassium and tissue mass as men At the same time they developed a tendency to beard growth The patients did not feel any better and there was as severe osteoporosis as in the group who did not receive anabolic steroids

Dr Kalbak Is arterial hypertension a side-effect of any importance in long term treatment with glucocorticoids?

Dr Quaade No steroid induced hypertension has never been a problem in the materials of which I have knowledge

With reference to osteoporosis I would like to mention that some workers have attempted to treat it with vitamin D as there is an inhibition of calcium absorption and an increased excretion of calcium in the urine

Dr Lorenzen The majority of experimental studies would suggest that glucocorticoid induced osteoporosis is due to an inhibition of the formation of bone matrix including various chrondroitin sulphates It is therefore not to be expected that vitamin D would be capable of counteracting osteoporosis

REFERENCES

1 Cope C L Adrenal steroids and disease Pitman Medical Publishing Co London pp 313–343 1964
2 Dubois E L Lupus erythematosus A review of the current status of discoid and systemic lupus erythematosus and their variants McGraw–Hill Book Company London Toronto New York 1966
3 Spiro H M & Milles S S Clinical and physiological implications of the steroid induced peptic ulcer New Eng J Med 263 286–294 1960
4 Warrell D W & Taylor R Outcome for the foetus of mothers receiving prednisolone during pregnancy Lancet I 117–118 1968

CONCLUDING PI MA ' _

We have now reached the conclusion of thi s,ir;o ı.rı in Internal Medicine To summarize the ars ver t value of the glucocorticoids outside substitutio ı th. in certain diseases these hormones can chan.e the cr in a favourable direction This is particularly tr. haematology and nephrology

However for a very large group of disord.r ha c r surprising and depressing result that it is not possible to gi questions because of the lack of controlled investigations T fact that the glucocorticoids have been used for many years in a of patients all over the world The uncontrolled investigations and the studies which have been carried out confirm the impression of a tomatic effect but suggest that the glucocorticoids do not co ıngly ch nce either the duration or the prognosis of the diseases At the sarr t e experience of the side effects of the glucocorticoids has made it cl ar th. s ı eatmeı t may confer a new disorder on the patient and that this rew disorde in itself has a certain mortality rate

These findings should encourage us to look more closely at the indications for treatment with the glucocorticoids and to contribute to the elucidation of the range of indications by carrying out controlled investigations

Ib Loren en

INDEX TO SUBJECTS